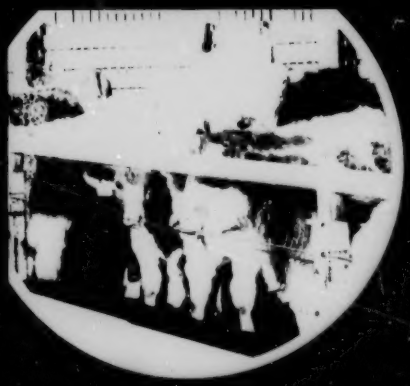

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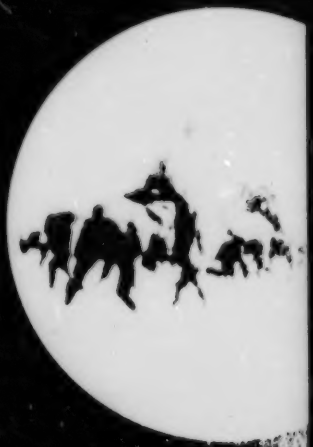

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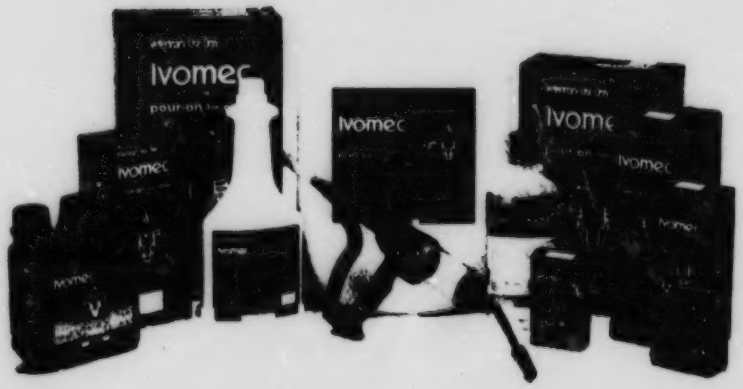

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ADVANCES IN BEEF CATTLE SCIENCE

VOLUME 1, 2001

K. A. BEAUCHEMIN and D. H. CREWS, JR.
EDITORS

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Preface

This volume of Advances in Beef Cattle Science contains, in chapter form, manuscripts presented at the 2001 National Beef Science Seminar (NBSS), held November 14-16, 2001, in Lethbridge, Alberta. The NBSS has rapidly grown to attract an international set of speakers, and the presentation topics included a cross-section of compelling issues in the beef industry, leading-edge science and new directions in production.

This book is organized into topic-oriented sections. While each manuscript contributes its own unique information, these sections remain important within the overall seminar theme: Emerging Issues and Opportunities in the Beef Industry. As the National Beef Science Seminar continues to mature, it is expected that this book will have companion volumes in the years to come.

The National Beef Science Seminar is hosted by the Agriculture and Agri-Food Canada Lethbridge Research Centre, the national headquarters for research in beef cattle science. The Centre mandate calls for ongoing communication with the beef industry across Canada. This seminar and trade show highlighted the best of beef science from the Lethbridge Research Centre, along with the best from the national and international beef research community.

Speakers at this year's seminar addressed the issues that affect the bottom line of producers today and the strength of the beef industry in the future. The presentations demonstrated the increasing importance of research to the economic and environmental sustainability of Canada's beef industry.

To ensure that research is relevant to producers and industry, who depend on access to the knowledge and the products flowing from research, the National Beef Science Seminar is part of an ongoing commitment of beef research efforts.

We thank our sponsors for their continued financial support, which is essential to maintaining a quality seminar. The development of this seminar and book is the result of the collaborative efforts of industry and government. We extend many thanks to those who may not be mentioned here, but were important links in the chain from data collection to scientific publication.

Karen Beauchemin and Denny Crews
November, 2001

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Today we have developed innovative strategies and tools to do just that. And we have grown to have the opportunity to work with some of the leading scientists in their field, worldwide.

We are proud to be a founding communications partner of the National Beef Science Seminar, and supporter of each one since. This Seminar is founded on the simple belief, that one of the real measures of scientific progress is when it is useful to the cattle producers on the front lines.

Our congratulations to the 2001 National Beef Science Seminar.

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Speakers:

Dr. Paul Kitching
Dr. John Nightingale (no manuscript submitted)

Session II - Sponsored by *Canadian Imperial Bank of Commerce (Lethbridge)*

Speakers:

Dr. Tim McAllister
Dr. Merle Olson
Dr. Tim Lysyk

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Intensive Animal Agriculture and Disease Control: Foot-and-Mouth Disease

Paul Kitching

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Key Points

- Foot-and-Mouth Disease can spread extremely quickly in areas of high animal density.
- The traditional methods of marketing sheep within the United Kingdom predisposed the rapid spread of the disease throughout most of the country.
- Once the disease was established, a large amount of resources were required to bring it under control.
- Other Foot-and-Mouth Disease free countries could derive valuable insights into disease control by studying the UK experience.

Introduction

Foot-and-mouth disease (FMD) is a highly infectious viral disease of cattle, pigs, sheep, goats and wild cloven hoofed animals, characterised by fever, loss of production, and vesicles on the mouth, feet and teats; in young animals the virus attacks the developing heart muscle, resulting in death without other clinical signs. It is caused by a small RNA virus, within the family Picornaviridae; there are seven immunologically distinct serotypes of FMD virus, types O, A, C, Asia1, and SAT1, SAT2 and SAT3. It is considered to be the most contagious of all animal virus diseases, and is the single most important constraint to international trade in live animals and animal products.

The United Kingdom had been free of FMD since 1981, when there had been a single outbreak in a dairy herd on the Isle of Wight, off the coast of southern England. On Feb 19th, this year, pigs waiting slaughter in an abattoir in southern Essex, to the north of London, were identified by the on-duty veterinarian with feet lesions consistent with FMD, and this was confirmed positive the following day at the high security Institute for Animal Health laboratory, Pirbright. This laboratory is also the World Reference Laboratory for FMD, and apart from having the largest research group working on FMD, also maintains a library of FMD virus isolates collected from around the world during the last 60 years.

The virus strain was quickly identified by nucleotide sequencing to be the PanAsia strain of serotype O FMD virus, which was known to be present throughout most of Asia, and had recently caused new outbreaks in Japan (free since 1908), South Korea (free since 1934) and South Africa (this was the first outbreak of serotype O ever recorded), Mongolia and eastern Russia. The pigs affected in the abattoir had been held over the weekend before developing signs, and it was assumed that their farm of origin was the source of infection; however, tracing back showed no evidence of infection, and it was concluded that they had acquired infection in the abattoir. Other farms supplying pigs were then visited, and on the 23rd Feb, a farm in north east England, close to Newcastle was found with evidence of FMD;

on 24th Feb a movement ban on all FMD susceptible species was applied to the whole of the UK, which included markets, but even affected race meetings and dog shows. The affected farm contained over 500 adult, mostly cull sows and boars, and following clinical examination it was apparent that disease had probably been introduced as early as the beginning of February, as most of the pigs had lesions of approximately 10 days old. The farmer fed almost exclusively a diet of waste food (swill), collected from nearby schools, hospitals and restaurants. Regulations relating to swill feeding make it compulsory to boil all waste food before feeding to pigs, but this is difficult to enforce.

FMD transmits following the movement of infected animals, the feeding of animal products contaminated with FMD virus, by contact with mechanically carried FMD virus (on vehicles, clothes, hands, instruments, etc.) or as an aerosol produced by infected animals. Persistently infected (carrier) cattle can also precipitate new outbreaks, although this is rare. It is likely that the pigs had been infected from the swill feed, and subsequent seizure of illegally imported animal products by the customs authority supported the hypothesis that this farm was the index case. Infected pigs produce a large amount of aerosol virus, up to 3000 times more than infected sheep or bovines, and immediately neighbouring farms were examined for evidence of disease. A number of nearby farms had been exposed to the virus, and one in particular, on which there were infected sheep and cattle, had earlier sold sheep through two markets to a farm in south west England, in Devon. When this farm was visited, it also was infected with FMD. Tracing back through the markets revealed a very large number of potential contacts, and visiting these contact premises quickly exhausted the veterinary resources of the Ministry of Agriculture (MAFF, now called DEFRA). Only 200 veterinarians were employed by MAFF, and vets that had once visited an infected farm could not, for disease security reasons, go on another farm for 3 days. However, the potential animal contacts from the markets, and their subsequent contacts, numbered many thousands, because of the methods of sheep trading used by the animal dealers, whereby some animals could change owner up to seven times in as many days.

FMD outbreaks then started appearing throughout the country, particularly in the west of England and southern Scotland and Wales. Disease had also spread by the movement of infected sheep to Northern Ireland, the Republic of Ireland, France and the Netherlands.

Control of the outbreaks was taken over from MAFF by the Chief Scientist, supported by four teams of mathematical modellers and other involved groups, who reported directly to the Prime Minister's Office. The slaughter policy was extended to all neighbouring farms and those within a 3 km radius of the infected farm; slaughter of the infected farm was to be completed within 24 hours of diagnosis, and neighbouring farms within 48 hours, and this became the responsibility of the Army.

Clinical disease in cattle and pigs is relatively easy to diagnose, but FMD in sheep is frequently mild, and can easily be confused with other common conditions, such as footrot, orf and non specific mouth ulcers. The majority of suspect cases were in sheep, and while samples were not collected from all of the suspect farms, of those that were submitted for laboratory confirmation, many were negative. The rate of transmission of FMD in sheep is also much lower than in cattle or pig herds, so that the potential for infected sheep flocks to transmit the virus as an aerosol to neighbouring farms is considerably lower (Sorensen et al., 2000). Consequently many uninfected animals were slaughtered, which added to the problem of carcase disposal. During the last major outbreak of FMD in the UK, in 1967/68, approximately 2500 farms were diagnosed infected and 500,000 animals were slaughtered; during the current outbreak approximately 1800 farms have been diagnosed infected, and over 4,000,000 animals have been slaughtered. The policy of slaughtering neighbouring farms and those within a 3 km radius was modified once it was shown that this particular strain of type O virus did not spread

significantly as an aerosol (Donaldson et al., 2001), and that most transmission that occurred after the movement ban was a consequence of illegal movements, direct contacts, or farmers not applying strict disinfectant procedures when visiting other livestock - farmers in the north of England frequently have several livestock holdings on different premises. The use of vaccination was considered, but would not have significantly altered the disease distribution or the final outcome, and was likely to confuse subsequent re-establishment of disease free status.

The total cost of the outbreak has not been calculated, but including the effect on tourism, will exceed $30 billion CDN.

Discussion

Resources

While the lessons to be learned from the recent FMD outbreak in the UK await to be fully documented in a Government enquiry, there were a number of problems which arose during the course of the outbreak which would have been better addressed by contingency planning before the event. Some of these were predictable, but most only became apparent once the outbreak had started. Most important was the availability of resources such as veterinary staff, valuers and slaughterers. UK, like all other European countries had been reducing its Civil Service numbers, including those employed by MAFF, and while it was always considered that an outbreak of FMD was possible, it was never envisaged on the scale that occurred. The concept that the first case would immediately be identified, and subsequent spread prevented, was clearly wrong; as was the assumption that other European countries would be infected before the UK, and that there would therefore be ample warning of its possible introduction. But no government in the world would have been able to maintain the manpower resources required, for an event that might never happen. Arrangements were in place to bring in veterinarians trained in disease control from around the world, but the manner in which the disease became established throughout the UK before its presence was even realized, made it impossible to bring in the necessary help sufficiently quickly.

The Sheep Industry

Although the disease started in pigs, its spread and establishment in the sheep population also delayed and confused the control programme, because of the difficulty of recognizing clinical signs in sheep. Farmers did not immediately recognize infection, and even after the declaration of the outbreak, many were not taking sufficient precautions when moving between flocks, because they thought their own animals were free of FMD. Consequently when disease was recognized, either in the sheep, or because it had spread to in-contact cattle or pigs, the virus was well established in a number of flocks owned by the same farmer, and possibly in neighbouring farms; on quite a large number of occasions, the disease had been present for many weeks, having been introduced before the movement ban. Added to this was the very large volume of sheep movements that were routinely taking place at that time of year. There were also a large number of sheep markets taking place, and it is normal for farmers to inspect the mouths and teeth of any sheep they intended to buy, which is an ideal way of spreading infection, and contaminating the farmers themselves. There were even instances of the handlers in the markets taking infection back to their own farms. Most of the transactions that occurred in the markets were recorded, but those occurring outside the market, for instance in the car park, were not registered, which made tracing even more difficult, particularly as the sheep were not individually identified. There was legislation put in place following the outbreaks of swine vesicular disease in the 1980's in the UK

that prevented the movement of pigs off a farm for 21 days after the introduction of any additional pigs from another premise. This did not apply to sheep, so it was frequent to discover that sheep had been moved from one farm to another over a relatively short time period, particularly through the premises of the sheep dealers.

Swill Feeding

The FMD virus had almost certainly been introduced with infected meat, imported illegally. There had been an outbreak of classical swine fever (hog cholera) during the previous year in the UK, also thought to have been introduced in infected meat products, which had already alerted MAFF to the dangers of this route of introduction. The volume of illegal movement of all products across international borders is impossible to quantify, but is likely to be extremely large, involving drugs, cars, even people, and the ability of any country to control it is limited by staff resources and the desirability not to disrupt legal movements. It is likely that no country can prevent the occasional introduction of a foreign animal pathogen, the only defence is to prevent it making contact with a susceptible species. The feeding of waste food to pigs is an obvious method by which this contact can take place, in spite of any legislation in place to render the material sterile by boiling or other treatment. In the UK, approximately 1% of the pigs were fed some swill in their diet, making it a marginal market of little economic significance to the pig industry. However, the consequences of the introduction of a disease such as swine vesicular disease, hog cholera, pseudo rabies or FMD affects all producers, not only pig producers - many more could be added to this list of pathogens such as strains of salmonella, arteriovirus, parvovirus, circovirus. The European Union has now banned the use of swill in pig feed throughout Europe.

Carcass Disposal

Because of the large numbers of animals being slaughtered, carcass disposal became a prominent issue in the media, and the pictures of large piles of dead animals clearly affected the decision of many tourists to visit the UK. While it remains the duty of the Government enquiry to establish whether the size of the cull was necessary, the consequence of the control strategy employed, and the use of the army to carry it out, resulted in an accumulating number of carcasses which could not be immediately buried or burnt. Because of environmental considerations such as proximity to housing and water catchment areas, it was not usually possible to bury or burn carcasses on the infected farms. Many were transported in leakproof vehicles to rendering plants, but these were quickly overwhelmed. Other problems encountered included: cattle over 30 months of age could not be buried because of the possible contamination of the ground with the agent of BSE, leakage from burial sites could contaminate local streams, funeral pyres could give off dioxins as well as acrid smoke. Together these considerations made decisions on whether to bury, burn or transport extremely difficult and complex, involving many different agencies.

Public Footpaths

The decision to close all footpaths across non urban land also severely affected the tourist industry, making it pointless for visitors to take holidays in some of the most scenic areas of the UK. A risk assessment had been carried out as to the dangers associated with free movement of people across land on which there could be susceptible animals, and while the risk of mechanically carrying the virus from an infected farm would have been small, the decision was taken. It can be appreciated that farmers

worried about the virus getting on to their land would be very concerned about strangers using footpaths in proximity to their animals.

Wildlife

At no time during the outbreak was there any evidence for the involvement of susceptible wildlife species, such as deer or feral pigs. The diagnostic laboratory received samples from farmed deer close to infected farms, and from deer killed by hunters or in road accidents, all were negative. This is consistent with FMD outbreaks in other regions of the world, except southern Africa, where the African buffalo is often associated with outbreaks in domestic stock. Generally, if wildlife are affected, they are unable to maintain the disease because of low stocking density or lower susceptibility, and the virus dies out - even if they were affected it would be inadvisable to attempt control because it would likely scare the wildlife involved out of the area, and thereby possibly introduce FMD into a previously free area.

Vaccination

Considerable discussion took place concerning the use of vaccination, and vaccine was formulated from the bank held in the UK for use in the north of England. The situation was different from that in the Netherlands where vaccination was used, because unlike the Dutch outbreak, which was focal, and could be surrounded by a barrier of vaccinated animals, the outbreak was well distributed around the UK before FMD was even recognized, making the choice of where to vaccinate impossible. The total sheep population exceeded 20 million, and cattle over 10 million, and because the distribution of the disease was not known, vaccination could have actually spread the disease. None of the animals in the UK had previously been vaccinated, and therefore would not have become protected for at least 5 days; if already infected animals were vaccinated, there was the danger of needle spread of the virus, as well as the danger that bringing the animals together for vaccination would encourage contact spread. Vaccination would not prevent disease in infected animals, and even those animals that were vaccinated would not be saved from infection should they have subsequently contacted active virus. It is a characteristic of FMD virus that it will persistently infect ruminants, following recovery from clinical disease, or even animals which have been protected by vaccination; cattle can remain carriers for up to 3 years, and sheep for up to 9 months. There is a small risk that these infected animals can precipitate new outbreaks of disease. Not only does vaccination not prevent infection, but the duration of immunity is generally less than 6 months, and it will not completely protect all vaccinated animals, depending on the level of live virus challenge they are exposed to. Pigs cannot be fully protected by vaccination, as if one of a group does develop disease, it will overwhelm the immunity of the others. One option was to vaccinate the cattle coming out of winter accommodation in the north of England, onto land on which there may have been infected sheep. If they had become infected, they would have consumed considerable resources in their disposal. The idea was discarded after the Food Standards Agency insisted (although later changed its mind) that milk from vaccinated animals would have to be labelled; the farmers rightly said that this would reduce the value of the milk, and could even prevent its sale. They were also concerned that they would have to slaughter their animals because they were vaccinated, and if that was likely, they preferred to have the compensation paid at once.

Re-establishment of FMD Free Status

The decision to slaughter vaccinated animals, as occurred in the Netherlands was determined by the need to re-establish freedom from FMD so that trading in live animals and animal products could be resumed. Free status can be obtained 3 months after the slaughter of the last infected animal, or the

last vaccinated animal, whichever is the later, in addition to a surveillance programme to show that the disease has been eradicated. The presence of vaccinated animals makes this more difficult, because of the possibility of carrier animals, and the problem of vaccinated animals having antibodies to FMD virus. If vaccinated animals are not slaughtered, a minimum of a year is required before the country can be recognized as disease free. The UK is currently undertaking a serum survey, testing up to 100,000 sera a week to prove to its trading partners that it has the disease under control, and ultimately eradicated.

Implications

At the time of writing (July 2001), sporadic outbreaks of FMD are still occurring in the UK. Most of these had been hidden by their association with sheep. This situation is quite normal in any large outbreak, and can be expected to cease over the next few months. Many questions have been raised concerning the management of the control programme, some of which have been discussed in this paper, and an enquiry will undoubtedly take place. The results of the enquiry will be of considerable interest and value to all FMD free countries, particularly those with an economically important and flourishing export trade in live animals and animal products.

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Escherichia coli O157:H7 Transmission and Control

Susan Bach and Tim McAllister[1]

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Key Points

- *E. coli* O157:H7 remains a significant human pathogen since its initial description in 1982.
- Cattle, both dairy and beef, represent a primary reservoir of *E. coli* O157:H7.
- *E. coli* O157:H7 can survive in the environment for extended periods of time.
- Effective control of *E. coli* O157:H7 requires reducing the frequency and intensity of *E. coli* O157:H7 fecal shedding by cattle in addition to targeting environmental sources of the organism.
- Effective methods to control *E. coli* O157:H7 in cattle have as yet not been identified.

Introduction

In the 19 years since its initial description, *E. coli* O157:H7 has become an important public health problem worldwide (Riley et al., 1983; Griffin and Tauxe, 1991). Given the magnitude and severity of recent outbreaks of *E. coli* O157:H7 infection, there is an urgent need to reduce the human health hazard caused by this pathogen. Foods of bovine origin have been implicated in numerous outbreaks and epidemiological investigations have shown that cattle are a major reservoir of *E. coli* O157:H7 (Wells et al., 1991; Hancock et al., 1994). The ability of the organism to survive in feed, water, soil and manure has important implications for its maintenance in cattle herds and contamination of water supplies and crops (Lynn et al., 1998; Fenlon et al., 2000; Buchko et al., 2000a, 2001; Hancock et al., 2001). Control measures that reduce or eliminate *E. coli* O157:H7 in the feedlot will not only reduce foodborne illness but will have a substantial impact on risks of transmission of the organism into the environment.

In this review, we present sources of *E. coli* O157:H7 and examine the incidence of *E. coli* O157:H7-associated infection since the emergence of the organism nearly two decades ago. Transmission of *E. coli* O157:H7 and potential methods for its effective control will also be addressed. The significance of *E. coli* O157:H7 as a human pathogen underlines the importance of establishing effective strategies to minimize numbers of *E. coli* O157:H7 on the farm.

History and Origins of *E. coli* O157:H7

Escherichia coli O157:H7 was first identified as a human pathogen following two outbreaks in the United States of gastrointestinal illness associated with undercooked hamburger patties (Riley et al.,

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1983; Wells et al., 1983). Whereas most *E. coli* strains are benign inhabitants of the gastrointestinal tract of humans and other warm-blooded animals, pathogenic *E. coli* strains exist which are associated with distinct syndromes of diarrheal disease (Levine, 1987). Those strains that produce Shiga-like toxins (SLT) or verotoxins (VT), and are associated with hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome in humans, are referred to as enterohemorrhagic *E. coli* (EHEC) (Kaper, 1994). The predominant EHEC serotype associated with food-borne illness, particularly in North America and the United Kingdom, is *E. coli* O157:H7. However, non-O157 EHEC serotypes, particularly serogroups O26, O111, O128 and O103, have also been implicated in sporadic enteric infections and possess outbreak potential (Johnson et al., 1996; Bettelheim, 2000).

It has been proposed that *E. coli* O157:H7 evolved from *E. coli* O55:H7, an enteropathogenic strain of *E. coli* that has long been associated with diarrhea in infants worldwide (Whittam et al., 1993; Wachsmuth et al., 1997; Mead and Griffin, 1998). Characteristic of other enteropathogenic *E. coli* strains, *E. coli* O157:H7 can adhere to epithelial calls and produce attaching and effacing (AE) lesions. It is thought that *E. coli* O55:H7, already possessing a mechanism for adherence to intestinal cells, acquired SLT genes from *Shigella*, and the O157 antigen through horizontal gene transfer and recombination (Mead and Griffin, 1998; Whittam et al., 1993).

The complete sequence of the *E. coli* O157:H7 genome has recently been determined and has contributed to our knowledge about the evolution of this organism. The closest relative of *E. coli* O157:H7 turns out to be the laboratory strain *E. coli* K-12 MG1655. These two strains (*E. coli* O157:H7 and K-12) share a common 4.1 million-base pair backbone with almost identical gene order. Scattered within each genome are hundreds of sections of DNA that are unique to one or the other strain, with much of the DNA in the O and K islands having been acquired through horizontal gene transfer. From the extent of the genetic differences between the two strains, it has been estimated that *E. coli* O157:H7 and *E. coli* K-12 shared a common ancestor about 4.5 million years ago (Perna et al., 2001).

Sources of *E. coli* O157:H7

Cattle as a Reservoir of *E. coli* O157:H7

Since the first two outbreaks of *E. coli* O157:H7 infection in 1982, a bovine source of *E. coli* O157:H7 has been implicated (Riley et al., 1983; Wells et al., 1983). Initial studies showed a low prevalence of *E. coli* O157:H7 in the feces of cattle (Hancock et al., 1994; Faith et al., 1996). In one study, the prevalence of *E. coli* O157:H7 was reported to be 0.28% in dairy cattle and 0.71% in beef cattle with a herd prevalence of 8.3% and 16% respectively (Hancock et al., 1994). More recent studies, however, have reported much higher levels. Chapman et al. (1997a) isolated *E. coli* O157:H7 from the feces of 15.7% of cattle (752/4800) over a 1-year period. The monthly prevalence ranged from 4.8 to 36.8%, with the highest prevalence occurring in the spring and late summer (Chapman et al., 1997a). Van Donkersgoed et al. (1999) isolated *E. coli* O157:H7 from 7.5% of fecal samples collected from cattle at slaughter, and the prevalence of *E. coli* O157:H7 in fecal samples from yearling cattle was higher than in samples from cull cows (12.4% vs. 2.0%). Even more recently, Elder et al. (2000) reported a prevalence of 28% (91 of 327 animals) for *E. coli* O157:H7 in fecal samples taken from slaughter cattle during July and August. In surveys of feedlots in the United States, *E. coli* O157:H7 was isolated from 63 to 100% of feedlots, indicating widespread distribution of *E. coli* O157:H7 in cattle operations (Dargatz et al., 1997; Elder et al., 2000).

The increased prevalence of *E. coli* O157:H7 reported in recent studies has likely arisen from the use of more sensitive isolation methods. A 10- to 100-fold increase in the sensitivity of detection of *E. coli* O157:H7 from bovine fecal samples has been reported through the use of immunomagnetic separation (Chapman et al., 1994). Geographic location also has an impact, with higher prevalences of *E. coli* O157:H7 being reported in the northwest USA and northern England (Sheffield), than in other areas (Chapman, 2000). Other variables include season, number, frequency and timing of sampling, in addition to transport and storage of samples (Van Donkersgoed et al., 1999; Elder et al., 2000).

Numerous studies have reported isolation of *E. coli* O157:H7 from healthy beef and dairy cattle, showing that cattle are asymptomatic carriers of the organism (Wells et al., 1991; Hancock et al., 1994; Zhao et al., 1995). Fecal shedding of *E. coli* O157:H7 by cattle is intermittent in nature, characterized by short periods with a relatively high prevalence of excretion separated by longer periods of reduced or undetectable shedding (Wells et al., 1991; Besser et al., 1997). Reported levels of *E. coli* O157:H7 shed by cattle have been ranged from 10^2 to 10^5 CFU/g feces (Zhao et al., 1995). Shedding of *E. coli* O157:H7 by cattle tends to be seasonal, peaking between May and September (Hancock et al., 1994; Van Donkersgoed et al., 1999). This seasonal pattern may contribute to the similar seasonal pattern of *E. coli* O157:H7-associated food borne illness in humans (Ostroff et al., 1989).

Other Animal Sources of *E. coli* O157:H7

Although epidemiological investigations of animal populations have concentrated on the bovine reservoir of *E. coli* O157:H7, other animal hosts have also been identified (Beutin et al., 1996). Chapman et al. (1997a, 1997b) reported isolation of *E. coli* O157:H7 from 2.2% of sheep and 0.4% of pigs at slaughter, but the organism was not detected in samples from 1000 chickens. In other studies, the prevalence of *E. coli* O157:H7 in sheep varied from 0 to 31%, indicating that sheep may play as significant a role as cattle in harbouring *E. coli* O157:H7 (Kudva et al., 1996; Heuvelink et al., 1998; Fegan and Desmarchelier, 1999).

Trevena et al. (1996) suggested that horses and dogs may be vectors of *E. coli* O157:H7, due to the close contact between farm animals, companion animals and humans. Levels of detection of *E. coli* O157:H7 of 1.1% for horses and 3.1% for dogs were reported by Hancock et al. (1998). The organism has also been isolated from wild deer and the feces of wild birds (Rice et al., 1995; Keene et al., 1997; Wallace et al., 1997; Hancock et al., 1998), suggesting the possibility of transfer of *E. coli* O157:H7 between animals and birds that share the same habitat as cattle.

The high variability of prevalence of *E. coli* O157:H7 among cattle suggests the possibility of a reservoir of *E. coli* O157:H7 external to cattle (Hancock et al., 1998). The intermittent and seasonal nature of *E. coli* O157:H7 shedding by cattle may indicate that cattle are a vehicle for transmission of the organism, as opposed to a reservoir in which the organism is maintained for long periods of time (Hancock et al., 1998). Reports of *E. coli* O157:H7 in other animals and birds confirm the existence of non-bovine animal reservoirs of *E. coli* O157:H7.

Environmental Sources of *E. coli* O157:H7

Johnson et al. (1999) noted that there are many possible habitats for *E. coli* O157:H7 in the farm environment, including manure heaps, ponds, dams and wells, barns, calf hutches, straw and other bedding, feed and feed troughs, water and water troughs, farm equipment, ground and pasture, and

watercourses. Once present in the environment, the organism can be transferred to other sites by rainwater, wind, and removal and spreading of manure, as well as by animals and humans.

Feed. *Escherichia coli* O157:H7 was isolated from 6.3% of feed samples obtained from a dairy farm in Wisconsin (Shere et al., 1998). Growth of the organism has been demonstrated in a variety of wet grain mixtures and some silage-based mixtures *in vitro*, at temperatures similar to those which occur on a farm during the summer months (Lynn et al., 1998).

Poorly fermented laboratory silage has been shown to support the growth of *E. coli* O157:H7 (Fenlon and Wilson, 2000). Two strains of *E. coli* O157:H7 inoculated onto grass subsequently ensiled under conditions allowing for aerobic spoilage increased from 10^3 CFU/g to 10^7 CFU/g over 13 days at 20°C. Poor ensiling technique, which resulted in delayed fermentation, created an environment conducive to the growth of *E. coli* O157:H7. Fecal contamination of forage (e.g., from manure application) followed by inadequate ensiling may therefore factor significantly in the persistence of *E. coli* O157:H7 carriage among cattle (Fenlon and Wilson, 2000).

Work in our laboratory has shown that adequate ensiling will reduce the risk of *E. coli* O157:H7 contaminated forage. Barley forage was inoculated with 10^5 CFU/g of *E. coli* O157:H7 and 10^5 CFU/g of a mixture of three strains of *E. coli* Biotype 1. The inoculated forage was subsequently ensiled for a 42-day period at 22°C in laboratory scale mini-silos. *Escherichia coli* O157:H7 and *E. coli* Biotype 1 were both eliminated following15 days of ensiling. The persistence of *E. coli* O157:H7 was further reduced if a silage inoculant was added to the forage prior to ensiling. This would allow silage to be fed out shortly after ensiling with minimal risk of transmitting *E. coli* O157:H7 to cattle (Buchko et al., 2001).

The seasonality of fecal shedding of *E. coli* O157:H7 implies that environmental replication may play a key role in the ecology of *E. coli* O157:H7 in the farm environment. It has been shown that *E. coli* O157:H7 can replicate in a variety of feeds, thus amplification of low levels of *E. coli* O157:H7 may increase the possibility of further dissemination of the organism on the farm.

Water for Livestock. Several studies report isolation of *E. coli* O157:H7 from animal drinking water (Faith et al., 1996; Hancock et al., 1998; Buchko et al., 2000a). The organism has been found to survive in the sediments of microcosms simulating cattle water troughs for periods exceeding 6 months, and the contaminated microcosms effected colonization of 10-week-old calves with *E. coli* O157:H7. Thus, water troughs can serve as environmental reservoirs of *E. coli* O157:H7 and contribute to subsequent infection of cattle (Lejeune et al., 2001).

Evidence exists that the means by which animal drinking water becomes contaminated may be oral rather than fecal. *Escherichia coli* O157:H7 was detected in drinking water obtained from covered water tanks equipped with ball-water ports (Shere et al., 1998). Fecal contamination of that water system was unlikely, given that physical depression of the ball in the port by the animal was necessary for receipt of water. The possibility of oral contamination of animals' drinking water is further supported by the recovery of *E. coli* O157:H7 has been recovered from the tonsils (Cray and Moon, 1995) and saliva (Buchko et al., 2000a) of cattle. Subsequent recovery of *E. coli* O157:H7 in the feces of cattle drinking water in which *E. coli* O157:H7 was known to be present suggests cattle may be susceptible to infection via drinking water (Shere et al., 1998).

Van Donkersgoed (2000) isolated *E. coli* O157:H7 from water troughs in feedlots, in addition to incoming water sources. The *E. coli* O157:H7 isolated from the source water, water troughs and feces of cattle were different subtypes, suggesting contamination of the water by a source of *E. coli* O157:H7 other than cattle feces. In addition to this, isolation of similar subtypes from feed, water and feces also implied transmission of the organism among these sources (Van Donkersgoed, 2000).

It is evident that water troughs may serve as long-term reservoirs of *E. coli* O157:H7. Survival of *E. coli* O157:H7 in water for extended periods of time, in addition to the potential of contaminated water as a point source of infection, emphasize the importance of the control of *E. coli* O157:H7 in animal drinking water.

Manure. There have been conflicting reports regarding the relationship between application of manure to grazing land and the prevalence of *E. coli* O157:H7 in cattle. A tentative association was found between the application of slurry to grazing land and the *E. coli* O157:H7 infection status of the grazing herd (Hancock et al., 1994). Subsequent studies, however, reported no association between manure application to pastures or cattle forage crops and *E. coli* O157:H7 fecal shedding by cattle (Garber et al., 1995; Hancock et al., 1997b). Conversely, a more recent study indicated that current waste management practices such as spreading manure on pastureland may increase the carriage rate of *E. coli* O157:H7 in herds (Bolton et al.1999).

Survival of *E. coli* O157:H7 in manure and manure slurry has been observed under various experimental and environmental conditions. Kudva et al. (1998) found that *E. coli* O157:H7 from experimentally inoculated sheep survived for 21 months in manure incubated under environmental conditions. In aerated ovine and bovine manure piles, the organism survived for 4 months and 47 days respectively. In bovine feces inoculated initially with 10^5 CFU/g, *E. coli* O157:H7 survived for 70, 56 and 49 days, at 5, 22 and 37°C, respectively (Wang et al., 1996). Fukushima et al. (1999) found that *E. coli* O157:H7 inoculated at 10^3 CFU/g survived in bovine feces for 2 to 14 weeks at 5°C, 1 to 18 weeks at 15°C, and 3 to 12 weeks at 25°C.

Contamination with bovine manure has been suggested in several cases of human infection associated with non-bovine food products. Inadequately washed vegetables were implicated as the source of *E. coli* O157:H7 infection following isolation of the pathogen from a garden fertilized with manure (Cieslak et al., 1993). The use of manure as fertilizer could explain foodborne outbreaks of *E. coli* O157:H7 associated with unpasteurized apple cider, potatoes, radish sprouts and lettuce (Morgan et al., 1988; Besser et al., 1993; Chapman et al., 1997b; Ackers et al., 1998; Michino et al., 1999). Manure may be a risk factor for transmitting *E. coli* O157:H7 to produce grown on manure-fertilized soil and may serve as a source for the maintenance of *E. coli* O157:H7 among cattle herds (Chapman et al., 1997b; Chart, 1998).

Given that *E. coli* O157:H7 can survive in manure and manure slurry for extended periods of time, proper manure management is of utmost importance in preventing the spread of this organism to the environment, food and animal crops, and back to cattle. Composting is an effective method for eliminating pathogens such as *E. coli* O157:H7 from manure. Pathogenic organisms such as *Giardia* and *Salmonella* were eliminated from compost after 14 days, whereas *Campylobacter* and *E. coli* O157:H7 were eliminated after 7 days (Olson, 2000). While few pathogens are able to withstand the heat generated during the composting process, it is important that all parts of the compost pile reach and maintain 60°C.

Drying has also been reported to be an effective strategy for reducing the presence of pathogens in manure (Pell, 1997).

Soil. Inoculated at a rate of 10^6 CFU/g of soil, *E. coli* O157:H7 survived in loam and clay soils for 25 weeks and in sandy soil for 8 weeks (Fenlon et al. 2000). The organism was detected for up to 7 d after application in the top 2.5 cm of the soil, and on the grass of grass plots inoculated with dairy cattle slurry at an application rate of 660 CFU/m^2. Approximately 2% of the initial inoculum of *E. coli* O157:H7 was transported to the deeper layers of the soil. Transport to drains was mainly associated with rainfall, which occurred 3 and 7 days after slurry application, leading to a cumulative loss of 7% of the applied *E. coli* O157:H7 (Fenlon et al., 2000).

Maule (2000) used model systems to determine the persistence of *E. coli* O157:H7 in soil cores. Soil cores containing rooted grass resulted in the greatest survival of the organism, with viable numbers declining from 10^8 CFU/g soil to between 10^6 and 10^7 CFU/g soil after 130 days at 18°C.

Tillage practice, soil type and method of pathogen delivery were each found to affect vertical transport of *E. coli* O157:H7 in soil (Gagliardi and Karns, 2000). Steady rainfall was applied to soil cores treated with manure inoculated with *E. coli* O157:H7. The presence of manure enhanced the survival of *E. coli* O157:H7 in intact (no-till) soils as opposed to disturbed (tilled) soils. High levels of recovery and low leachate levels of *E. coli* O157:H7 from clay loam soils, as compared to silt loam or sandy loam soils, indicated that growth of *E. coli* O157:H7 could occur if leaching losses were minimal. The research indicated that the organism could travel vertically through the soil for more than two months after initial application, if soil pores do not become clogged, possibly resulting in contamination of food and water (Gagliardi and Karns, 2000).

Barker et al. (1999) found that *E. coli* O157:H7 was able to survive and replicate in *Acanthamoeba polyphaga*, a common environmental protozoan. Those authors suggested that a dynamic and mutually beneficial interaction between *E. coli* O157:H7 and *A. polyphaga* existed in laboratory microcosms. In some instances, *E. coli* O157:H7 was digested as a food source by the protozoa but in others, the bacteria multiplied. Protozoa are widely distributed in soil, thus they may represent an important environmental reservoir of *E. coli* O157:H7 (Barker et al., 1999).

Flies. The increased presence of flies around cattle during the summer months represents a potential mechanism for the spread of *E. coli* O157:H7 among animals. A recent study was conducted on the potential of transmission of *E. coli* O157:H7 to fresh-cut apple tissues by fruit flies. Following contact with *E. coli* O157:H7, the fruit flies were contaminated with it both externally and internally, and the flies then transmitted the bacterium to uncontaminated apple wounds and increased the level of contamination (Janisiewicz et al., 1999).

Sasaki et al. (2000) detected *E. coli* O157:H7 at a level of 5×10^6 CFU in the crop of houseflies (*Musca domestica*) immediately after feeding on a bacterial preparation. Numbers of *E. coli* O157:H7 in individual drops of excreta were exceeded 10^4 after 1 h, and averaged 1.8×10^4 bacteria after 3 h. Excretion of *E. coli* O157:H7 continued to decline over the next 24 h period, indicating that houseflies are effective vectors of this organism. Isolation of *E. coli* O157:H7 from flies collected on dairy farms and feedlots has also been reported (Rahn et al., 1997; Hancock et al., 1998; Shere et al., 1998). Thus, the potential exists for dispersion of *E. coli* O157:H7 in the environment and to humans via contaminated flies.

Outbreaks of *E. coli* O157:H7 Infection

Following the identification of *E. coli* O157:H7 as the causative agent of human illness in 1982 (Riley et al., 1983), numerous outbreaks of illness associated with *E. coli* O157:H7 have been reported in Canada, the United States, Japan and the United Kingdom (Griffin and Tauxe, 1991; Besser et al., 1993; Ackman et al., 1997; Keene et al., 1997; Swerdlow and Griffin, 1997; Cody et al., 1999; Michino et al., 1999). An outbreak of *E. coli* O157:H7 in the United States in 1993 involving 700 people in four states resulted in dramatically increased awareness and concern about foodborne illness associated with *E. coli* O157:H7 (Bell et al., 1994). An outbreak in Lanarkshire, Scotland in 1996 resulted in 20 deaths and 496 cases of food poisoning (Liddell, 1997), and in Walkerton, Ontario in 2000, 167 confirmed cases of *E. coli* O157:H7 infection and six deaths (Bruce-Grey-Owen Sound Health Unit, 2000). The largest reported outbreak of *E. coli* O157:H7 occurred in 1996 in Sakai city, Japan, with a total of 9578 cases and 11 deaths (Michino et al., 1999). Increased recognition of *E. coli* O157:H7 as a cause of human illness has resulted in increased surveillance for *E. coli* O157:H7 and identification of the organism worldwide.

Geographic Distribution

E. coli O157:H7 infections have been reported most frequently in Canada, the United States and the United Kingdom, although illnesses due to *E. coli* O157:H7 have been reported in over 30 countries on six continents (Doyle, 1991; Griffin and Tauxe, 1991; Waters et al., 1994; Boyce et al., 1995; Chapman, 1995; Mead and Griffin, 1998; Nataro and Kaper, 1998). *Escherichia coli* O157:H7 is an important pathogen in Japan and Europe, whereas in countries such as Australia, Argentina, Chile and South Africa, non-O157 serotypes of EHEC are a more frequent cause of hemorrhagic colitis and hemolytic uremic syndrome (Bettelheim, 1998; Nataro and Kaper, 1998).

Infections due to *E. coli* O157:H7 appear to be more common in Canada than in the United States (Griffin and Tauxe, 1991; Boyce et al., 1995). Within the United States, outbreaks are apparently more common in the northern states than in the southern states (Griffin and Tauxe, 1991; Slutsker et al., 1997; Nataro and Kaper, 1998; Chapman, 2000). In Canada, reports of *E. coli* O157:H7 infection have been more frequent in the western provinces than in the east (Griffin and Tauxe, 1991; Nataro and Kaper, 1998). The highest annual provincial rates of verocytotoxin-producing *E. coli* (VTEC) infection for the period between 1992 and 1995 occurred in Prince Edward Island (10.2 cases per 100,000 persons), Manitoba (7.6 per 100,000) and Alberta (7.4 per 100,000) (Spika et al., 1998).

Rural *vs* Urban Environments

Michel et al. (1999) investigated the relative risks of VTEC infection associated with living rurally as opposed to in an urban area by examining the geographic distribution of VTEC infections in Ontario, Canada, between 1990 and 1995. Reported annual rates of VTEC infection were consistently higher in rural areas (5.4 per 100,000) as compared to urban areas (4.4 per 100,000). Spatial analyses revealed that cattle density was a significant indicator of VTEC infection in many regions of Ontario and it was suggested that an increased risk for VTEC infection existed among rural residents. The association of higher cattle density with increased risk of VTEC infection also likely explains the high incidence of VTEC infections in Prince Edward Island, where the cattle density (0.17 per hectare) approaches that of the regions of Ontario with the highest incidence of VTEC infection (0.2 cattle per hectare; Michel et al., 1999).

Seasonal Influence

Infections associated with *E. coli* O157:H7 appear to be more common during the summer months, with the majority of cases occurring during the months of May through September (Pai et al., 1988; Ostroff et al., 1989; Waters et al., 1994; Boyce et al., 1995; Slutsker et al., 1997; Coia, 1998; Mead and Griffin, 1998; Chapman, 2000). This seasonality parallels the increased prevalence of *E. coli* O157:H7 in cattle during the summer months (Hancock et al., 1994, 1997a; Boyce et al., 1995; Hancock 1997; Chapman et al., 1997a; Mechie et al., 1997; Lynn et al., 1998).

Incidence

It has been estimated that *E. coli* O157:H7 infection accounts for more than 20,000 cases of illness and results in as many as 250 deaths per year in the United States (Boyce et al., 1995; Coia, 1998). Results of the recently initiated Foodborne Disease Active Surveillance Network (Foodnet) by the Center for Disease Control (CDC) reported the incidence of *E. coli* O157:H7 in the United States in 1996 to be 3 per 100,000 population (CDC, 1996). In 1999, a total of 38 outbreaks of *E. coli* O157:H7 infection in the USA were reported to the CDC. The outbreaks were reported from 30 states and affected 1,897 people (CDC, 2001b). The Laboratory Center for Disease Control in Canada reported 1,334 cases associated with *E. coli* O157:H7 in 1995, which represents approximately 5 per 100,000 population (LCDC, 1995). In the United Kingdom, the highest rates of infection were reported in Scotland, with 9.8 cases per 100,000 reported in 1996 (Coia, 1998). Lower rates of infection (1.29 per 100,000 population) were reported in England and Wales in 1996 (Coia, 1998).

Modes of Transmission of *E. coli* O157:H7

Food Vehicles

Contaminated and improperly cooked ground beef is the principle vehicle implicated in *E. coli* O157:H7 infections (Borczyk et al., 1987; Bell et al., 1994;). Fecal contamination of carcasses during slaughter and processing is the likely manner by which *E. coli* O157:H7 is transferred to beef (Chapman et al., 1993a; Elder et al., 2000). Grinding the beef may further compound the problem by introducing the pathogen into the interior of ground meat patties where it is more likely to survive inadequate cooking (Boyce et al., 1995; Mead and Griffin, 1998). Ground beef often comprises meat from many carcasses, thus a few infected animals could potentially contaminate a large quantity of ground beef (Boyce et al., 1995).

Numerous foods other than ground beef have been linked to *E. coli* O157:H7 outbreaks (Table 1). Cross-contamination as a result of contact with bovine products or contamination with feces of wild or domestic animals has been suspected in the majority of these outbreaks (Armstrong et al., 1996; Mead and Griffin, 1998).

The levels of contamination in the food samples incriminated in *E. coli* O157:H7 outbreaks support the hypothesis of a low infectious dose (Neill, 1989; Armstrong et al., 1996). A community outbreak associated with beef burgers in Wales in 1993 was used to estimate the presence of *E. coli* O157:H7 in the burgers. Less than 2 organisms per 25 g sample of meat caused illness, implying that the infectious dose of *E. coli* O157:H7 could be as low as 10 organisms (Willshaw et al., 1994). The infectious dose of *E. coli* O157:H7 is also affected by variables such as stomach pH, food composition and host susceptibility (Cassin et al., 1998; Gannon, 1999).

Water

Outbreaks of *E. coli* O157:H7 infection have been associated with drinking water and with swimming (Swerdlow et al., 1992; Keene et al., 1994; Ackman et al., 1997; Paunio et al., 1999). In 1989, a large outbreak of *E. coli* O157:H7 infection occurred in Cabool, Missouri. This outbreak was associated with drinking water from an unchlorinated water supply (Swerdlow et al., 1992). The outbreak resulted in 243 cases of illness and four deaths. Epidemiological studies of three swimming-associated outbreaks suggest that fresh-water lakes were the source of *E. coli* O157:H7 infection. In those outbreaks, illness was presumably due to bathers swallowing small amounts of contaminated lake water (Swerdlow et al., 1992; Keene et al., 1994; Paunio et al., 1999). A recent outbreak of *E. coli* O157:H7 infection in Walkerton, Ontario was associated with contaminated municipal water, and resulted in 167 confirmed cases of *E. coli* O157:H7 infection and six deaths (Bruce-Grey-Owen Sound Health Unit, 2000).

Person-to-Person Transmission

During the large *E. coli* O157:H7 outbreak in the United States in 1993 due to contaminated beef burgers, approximately 11% of the cases were determined to be secondary infections (Bell et al., 1994). In Wales, 17% of the identified cases of *E. coli* O157:H7 infection were the result of contact with an index case. The household transmission rate of *E. coli* O157:H7 infection in Wales between 1994 and 1996 was estimated to be 7%, with children aged 1 to 4 years and adults aged 15 to 34 years most likely to contract infection from an index case patient through household contacts (Parry and Palmer, 2000). This pattern of transmission suggests that person-to-person spread is likely via fecal-oral routes, as opposed to foodborne transmission.

Person-to-person spread of *E. coli* O157:H7 has also been identified as the primary means of transmission of infection in day care centers (Swerdlow and Griffin, 1997). Person-to-person transmission has been identified in secondary cases of illness in a nursing home outbreak and has been implicated in an outbreak involving venison jerky (Ryan et al., 1986; Keene et al., 1997). It has been suggested that the spread of *E. coli* within families and in institutionalized settings may occur through means other than foodborne transmission (Doyle, 1991; MacDonald et al., 1996).

Bovine-to-Human Transmission

Three incidents of apparent direct transmission of *E. coli* O157:H7 from bovines to humans have been reported (Renwick et al., 1993; Synge et al., 1993; Rice et al., 1996). In Ontario, Canada, in 1992, a 13-month-old boy was hospitalized with hemorrhagic colitis following prolonged contact with calves on a dairy farm. In Scotland in 1993, a 15-month-old child who lived adjacent to a farm became ill with *E. coli* O157:H7 (Synge et al., 1993). It was suggested that the family dogs may have carried the organism into the household, resulting in the infection of the child. An additional case of *E. coli* O157:H7 infection acquired from livestock occurred in a 10-year-old boy who was actively involved in raising livestock (Rice et al., 1996).

In 1999, an outbreak of *E. coli* O157:H7 infection among visitors to a petting zoo resulted in 159 illnesses in Ontario, Canada (Warshawsky et al., 1999). Direct contact with farm animals as a result of school visits to farms resulted in outbreaks of *E. coli* O157:H7 infection in Pennsylvania and Washington in the spring and fall of 2000 (CDC, 2001a). Children were allowed to touch cattle, calves, sheep, goats, llamas, chickens and pigs. Lack of appropriate handwashing prior to eating and eating

while petting the animals may have been associated with infection (Warshawsky et al., 1999; CDC, 2001a).

Though direct transmission of *E. coli* O157:H7 from cattle and other animals to humans is rare, contact with livestock should be considered when infections due to *E. coli* O157:H7 occur in humans.

Control of *E. coli* O157:H7

In a simulation study, Jordan et al. (1999) showed that the pre-slaughter interventions with the greatest potential for reducing contamination of beef carcasses with *E. coli* O157:H7 are the vaccination of cattle against *E. coli* O157:H7 and the use of substances to reduce fecal shedding of *E. coli* O157:H7 by cattle. A study conducted by Elder et al. (2000) reported a positive correlation between fecal and hide prevalences of *E. coli* O157:H7 and subsequent contamination of carcasses with the organism. This implies that an opportunity for controlling *E. coli* O157:H7 exists at the bovine source. There is ample opportunity for cross contamination once the animal enters the packing plant, thus intervention strategies to reduce the levels of *E. coli* O157:H7 in the feces of cattle may subsequently reduce the cases of foodborne illness associated with this deadly pathogen.

Diet

Several studies have identified an association between diet type and fecal shedding of *E. coli* O157:H7 by ruminants (Kudva et al., 1995; Kudva et al., 1997; Diez-Gonzalez et al., 1998; Hovde et al., 1999), however other studies have found no such association (Magnuson et al., 2000; Tkalcic et al., 2000).

The effect of diet quality on fecal shedding of *E. coli* O157:H7 was investigated using experimentally inoculated sheep, and differences in diet quality resulted in distinctively different patterns of fecal shedding the organism (Kudva et al., 1995; Kudva et al., 1997). Sheep fed a diet high in fiber and low in nutrients (grass hay) shed *E. coli* O157:H7 in larger numbers and for longer periods of time than did sheep fed a diet low in fiber and high in nutrients (corn and pelleted alfalfa). With the two diets, fecal shedding of *E. coli* O157:H7 continued for 8 to 15 days and for 2 to 6 days, respectively (Kudva et al., 1997). These results suggest that feed management practices could reduce the frequency of *E. coli* O157:H7 fecal shedding by animals as well as the duration of time in which animals were positive for the organism.

In a study with cattle, those fed concentrate diets shed higher numbers of nonpathogenic *E. coli* in their feces than did those fed hay (Diez-Gonzalez et al., 1998). Further, the percentage of fecal *E. coli* that were acid tolerant was higher in concentrate-fed cattle than those fed hay. Hovde et al. (1999) determined that nonpathogenic coliforms from hay-fed cattle were more sensitive to acid shock than coliforms from grain-fed animals. However, in contrast to the findings of Diez-Gonzalez et al., the hay-fed cattle in this latter study shed *E. coli* O157:H7 in their feces for a significantly longer period of time (39 to 42 days) than did the grain-fed cattle (4 days), and irrespective of diet, the *E. coli* O157:H7 shed by both groups were equally acid resistant (Hovde et al. 1999).

Tkalcic et al. (2000) found no association between diet type and the incidence of ruminal populations of *E. coli* O157:H7 or fecal shedding of the organism in calves fed high concentrate or high roughage diets. Although proliferation of *E. coli* O157:H7 was favored in ruminal fluid from animals fed a high roughage diet compared with a high concentrate diet *in vitro*, a similar dietary effect was not

demonstrated *in vivo*. Magnuson et al. (2000) reported that forage-based growing diets and grain-based finishing diets did not affect the duration of times during which heifers cultured positively for *E. coli* O157:H7.

Interrelationships between feed quality, ruminal volatile fatty acid (VFA) concentrations and ruminal pH have been documented. Roughage diets increase ruminal pH and decrease VFA concentrations, whereas high concentrate diets decrease ruminal pH and increase VFA concentrations (Owens and Goetsch, 1988; Magnuson et al., 2000). Harmon et al. (1999) found no correlation, however, between daily fluctuations in ruminal VFA concentrations and ruminal or fecal *E. coli* O157:H7 numbers. Those researchers suggested that ruminal populations of *E. coli* O157:H7 remained low, and that replication occurred subsequent to introduction of the organism into the colon.

The significance of acid-resistant *E. coli* O157:H7 in bovine feces remains controversial. It has been suggested that because feeding grain results in a lower pH in the colon compared to feeding hay, this practise may increase the populations of acid-resistant *E. coli* in grain-fed versus hay-fed animals (Diez-Gonzalez et al., 1998). To date, research findings are discrepant regarding the effects of high concentrate and high roughage diets on fecal shedding of *E. coli* O157:H7 by cattle, and on consequent development of acid resistance of this organism (Diez-Gonzalez et al., 1998; Hovde et al., 1999; Tkalcic et al., 2000). These points, therefore, remain to be resolved.

We recently evaluated the effects of three different grain-based diets (85% barley, 85% corn or 15% whole cottonseed/70% barley) on fecal shedding of *E. coli* O157:H7 by experimentally inoculated steers (Buchko et al., 2000a). Although diet did not affect the numbers of *E. coli* O157:H7 shed by the steers, fecal shedding was detected in a significantly higher number of steers when the barley diet was fed, as compared with the other two diets (Figure 1). In that study, *E. coli* O157:H7 was recoverable from mouth swabs of the steers, as well as from feed, water and manure. This suggests that this organism can survive in the environment and the alkaline saliva of cattle long enough to enable transferral among cattle (Table 2).

The bovine gastrointestinal tract is a complex system, thus survival of *E. coli* O157:H7 in this system is undoubtedly influenced by multiple factors. Further study is necessary to determine the effect of diet on *E. coli* O157:H7 in the gastrointestinal tract of cattle before any recommendations can be made in terms of diet as a means of controlling this pathogen.

Fasting

Although some studies have shown that fecal shedding of *E. coli* increased in response to dietary stress, feed withdrawal was found to exert little effect on *E. coli* O157:H7 shedding (Kudva et al., 1997; Cray et al., 1998; Jordan and McEwen, 1998; Harmon et al., 1999; Buchko et al., 2000b). Fasting reduced ruminal VFA concentrations, with a resultant increase in the ruminal pH, but a correlation between VFA concentration and ruminal and fecal levels of *E. coli* O157:H7 were not observed (Harmon et al., 1999). Diet-stressed calves were more susceptible to *E. coli* O157:H7 infection than well-fed calves, however (Cray et al., 1998). In cattle actively shedding *E. coli* O157:H7, feed withdrawal had little effect on growth of the organism in the rumen, or on the numbers shed in the feces (Harmon et al., 1999). This suggests that fasting prior to slaughter does not increase fecal shedding of *E. coli* O157:H7 or the number of *E. coli* O157:H7 positive animals entering the food chain.

Probiotics

The protective intestinal microflora that establishes in animals soon after birth is inherently very stable, but it is subject to influence by certain dietary and environmental conditions. These include antibiotic therapy, overcrowding, changes in feed, handling, shipping and new environments. When animals are stressed, the balance between beneficial and undesirable organisms may become upset resulting in diarrhea, gastroenteritis and reduced feed intake and growth (Fox, 1988).

Fuller (1989) defined probiotics as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance". The concept of administering probiotics to animals involves the hypothesis that these beneficial microorganisms will combat the effects of stress and prevent pathogenic microorganisms from establishing in the intestine. The specific mode of action of probiotics may comprise a direct antagonistic effect against a specific group of organisms. Alternatively, suppression of bacterial numbers may be achieved by production of antibacterial compounds, or by competition for nutrients or adhesion sites. Probiotics may also alter microbial metabolism by affecting enzyme activity and/or by stimulating immunity, resulting in increased antibody levels and macrophage activity (Fuller, 1989).

Probiotic bacteria have proven effective for reducing the duration of ruminal carriage of *E. coli* O157:H7 in cattle. Eighteen bacterial isolates (*Proteus mirabilis* and 17 strains of *E. coli*) that produced metabolites inhibitory to *E. coli* O157:H7 were isolated from cattle not currently shedding *E. coli* O157:H7. The 18 bacterial strains were suspended in a 2% dilution of sterilized skim milk and administered orally to calves 2 d prior to their inoculation with *E. coli* O157:H7. Calves that received the probiotic bacteria carried *E. coli* O157:H7 ruminally for only 9-17 days, compared with 22-32 days in the control calves. Fecal shedding was reduced from 25-32 days in the control group to 14-19 days in those receiving the probiotic (Zhao et al., 1998).

Vaccination

Vaccination may be an effective intervention strategy for reducing carriage of *E. coli* O157:H7 by cattle. The goal of vaccination is to either reduce the susceptibility of cattle to colonization by *E. coli* O157:H7, or to decrease the duration of such colonization. In order for *E. coli* O157:H7 to be shed, it must first be able to survive in the gastrointestinal tract of cattle. Researchers believe that *E. coli* O157:H7 adhere to the wall of the large intestine by means of surface structures on its cell wall. A vaccine that induces production of antibodies against these surface structures could prevent adherence of the organism and result in its elimination from the gastrointestinal tract. Potter and Finlay (2000) reported that vaccinating cattle with two recombinant antigens, Tir and EspA, reduced fecal shedding of *E. coli* O157:H7 compared with unvaccinated control animals.

Bacteriophage Therapy

Bacteriophages, initially described in the early 1900's, have been used successfully to control human bacterial pathogens such as *Salmonella*, *Shigella* and *Staphylococcus* spp. (Barrow and Soothill, 1997; Alisky et al., 1998; Sulakvelidze et al., 2001). Treatment with bacteriophages has also been effective for controlling enteropathogenic *E. coli* infection in mice (Smith and Huggins, 1982), calves, piglets and lambs (Smith and Huggins, 1983); phage therapy was found to be most effective when administered prior to or concurrently with the infective bacterial challenge.

Three O157-specific bacteriophages were shown to lyse liquid cultures of *E. coli* O157:H7 at 4 and 37°C (Kudva et al., 1999). Culture aeration and high multiplicity of infection (MOI) were required for complete lysis; no single phage was able to completely lyse the culture. Full lysis of the bacterial culture was achieved during 8 h of incubation, with aeration, at 4 and 37°C when cultures were infected with all three phages at a multiplicity of infection (MOI) of 10^3 plaque forming units (PFU) per CFU. In non-aerated cultures, complete elimination of the bacteria required 5 d of incubation at 4°C.

Waddell et al. (2000) determined that the duration of fecal shedding of *E. coli* O157:H7 by calves inoculated with 3×10^9 CFU of the organism could be significantly reduced by administering 10^{11} PFU of each of six O157-specific bacteriophages to the calves 7, 6, 1 and 0 d prior to, and 1 d after inoculation. Shedding of *E. coli* O157:H7 by control calves (*E. coli* O157:H7-inoculated only) occurred for 6 to 14 d, whereas calves treated with the bacteriophages shed the pathogen for 6 to 8 d only.

Studies to date suggest that multiple bacteriophages administered in combination are more effective for eliminating *E. coli* O157:H7 than those given alone. Further, bacteriophage therapy is more effective when given before or with the infective bacteria, than subsequent to infection. These constraints may limit the usefulness of bacteriophage as a control measure against fecal shedding of *E. coli* O157:H7 by cattle (Smith and Huggins, 1982; Smith and Huggins, 1983; Kudva et al., 1999; Waddell et al., 2000).

Additional Control Measures

Other strategies for intervention in *E. coli* O157:H7 infections include administration of coumarins, sodium chlorate, prebiotics, synbiotics and colicins (Murinda et al., 1996; Duncan et al., 1998; Anderson et al., 2000; Gyles, 2000). Plant metabolites such as coumarins have been found to inhibit *E. coli* O157:H7 *in vitro*. Growth of *E. coli* O157:H7 was also inhibited under aerobic and anaerobic conditions by esculetin, umbelliferone and scopoletin (Duncan et al., 1998). The mechanism of inhibition of *E. coli* O157:H7 by coumarins is unknown, but coumarins are recognized as enzyme inhibitors and are known to act as antioxidants in biological systems (Duncan et al., 1998). These compounds are found in a wide variety of animal forages as well as in fruits and vegetables consumed by humans.

Anderson et al. (2000) found that chlorate exerted an inhibitory effect on *E. coli* O157:H7 in ruminal contents *in vitro*. A respiratory nitrate reductase, possessed by both *E. coli* O157:H7 and by some *Salmonella* spp., reduces chlorate to chlorite intracellularly, which results in cell death. The antimicrobial properties of chlorate were found to be greater at 5 mM than at 1.25 mM, and at pH 6.8 than at pH 5.6 (Anderson et al., 2000).

Colicins are bacteriocins produced by *E. coli*. These agents have exhibited inhibitory activity against *E. coli* O157:H7 *in vitro*, and may have potential for controlling *E. coli* O157:H7 in cattle (Murinda et al., 1996). Prebiotics (feed ingredients, such as inulin, that stimulate microbial growth) and synbiotics (combinations of probiotics and prebiotics) are other intervention strategies that may have potential for reducing fecal shedding of *E. coli* O157:H7 by cattle, but to date few reports have been published on the use of colicins, prebiotics and synbiotics in this application.

Environmental Control of *E. coli* O157:H7

Survival of *E. coli* O157:H7 for extended periods of time (weeks to months) in livestock production environments may enable transfer of the organism back to cattle through contaminated feed or water. This creates a cycle of infection allowing for *E. coli* O157:H7 to be maintained in cattle herds. Effective control of *E. coli* O157:H7, therefore, requires suppression at each point in the cycle of infection in order to reduce the spread of this pathogen (Figure 2). Minimizing contamination of water troughs and feed bunks together with proper manure management will contribute significantly to reducing the spread of *E. coli* O157:H7 to cattle, crops and water sources.

Implications

Presently, *E. coli* O157:H7 outbreaks and product recalls as a result of *E. coli* O157:H7 constitute one of the largest threats to the long term sustainability of the Canadian beef industry. Incidents such as Walkerton, as well as the recent product recalls by a number of beef processing plants, undermine consumer confidence and target the beef industry as a major point source of environmental contamination. Should the frequency of these events increase, damage to the Canadian beef industry could be virtually incalculable. If such trends are to be reversed, proactive steps must be taken to reduce fecal shedding of this bacterium by feedlot cattle and to reduce its prevalence and persistence in the environment. These steps will in turn reduce the number of *E. coli* O157:H7-positive animals arriving at packing plants and thus minimize foodborne illness associated with this human pathogen.

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Table 1. Foods other than ground beef associated with outbreaks of *E. coli* O157:H7 infection

Year	Location	Food	Reference
1985	UK	potatoes	Morgan et al., 1988
1986	Canada	unpasteurized milk	Borczyk et al., 1987
1987	UK	turkey roll	Salmon et al., 1989
1991	USA	apple cider	Besser et al., 1993
1993	UK	unpasteurized milk	Chapman et al., 1993b
1993	UK	yoghurt	Morgan et al., 1993
1994	USA	mayonnaise	Weagent et al., 1994
1995	USA	dry-cured salami	CDC, 1995
1995	USA	lettuce	Ackers et al., 1998
1995	USA	venison jerky	Keene et al., 1997
1995	UK	potatoes	Chapman et al., 1997b
1996	Japan	radish sprouts	Michino et al., 1999
1996	UK	meat products	Liddell, 1997
1996	USA/Canada	apple juice	Cody et al., 1999
1997	USA	alfalfa sprouts	CDC, 1997

Table 2. Recovery[z] of *E. coli* O157:H7 strain 3081 from mouth swabs of experimentally inoculated steers and their environment using Dynabead™ isolation following overnight enrichment in mTSB[y] at 37°C (Buchko et al., 2001a).

Diet[x]	Mouth swabs	Water	Water-trough biofilm	Feed[w]	Manure
Corn	7/60	1/10	1/10	0/10	10/10
Cottonseed	4/60	1/10	0/10	2/10	10/10
Barley	19/60	1/10[v]	2/10	3/10	10/10
Total positive	30/180	3/30	3/30	5/30	30/30

[z]Recovery is calculated as number of cultures positive for *E. coli* O157:H7/number of samples.
[y]mTSB = Trypticase Soy Broth amended with 20 μg/mL novobiocin.
[x]Diets contained 85% grain and 15% alfalfa silage (DM basis). The cottonseed diet comprised 15% cottonseed and 70% barley grain.
[w]Samples were collected from feed mangers 4 h after animal access.
[v]A non-3081 strain of *E. coli* O157:H7 was cultured.

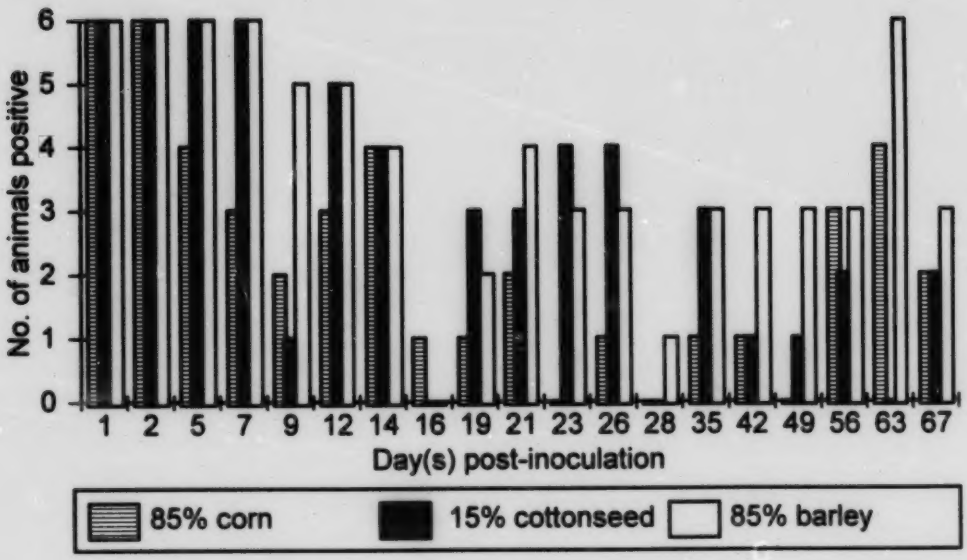

Figure 1. Effect of diet on isolation of *E. coli* O157:H7 strain 3081 from the feces of steers experimentally inoculated with 10^{10} CFU of *E. coli* O157:H7 strain 3081 ($n = 6$). The cottonseed diet also contained 70% barley grain. The forage component (15% DM basis) of each of the three diets was alfalfa silage.

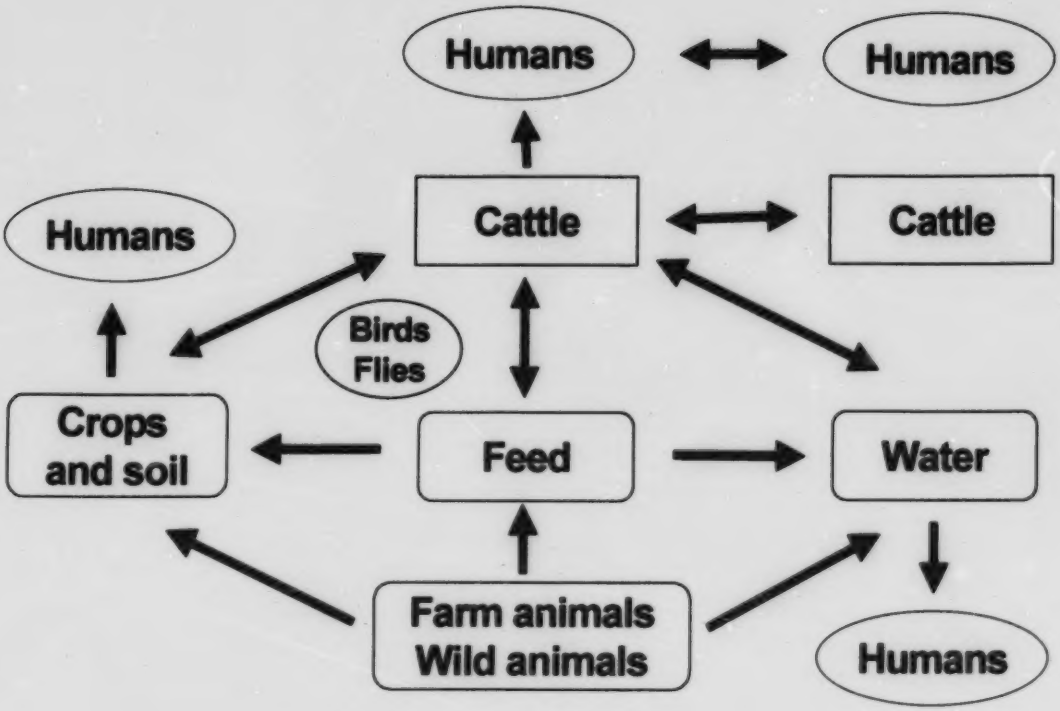

Figure 2. Transmissibility of *E. coli* O157:H7 in the environment giving rise to a cycle of infection that may enable maintenance of the organism in cattle herds. Effective control of *E. coli* O157:H7 requires control at each point in the cycle in order to minimize the incidence of foodborne disease associated with this human pathogen.

Giardiasis and Cryptosporidiosis in Cattle

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Key Points

- Giardia duodenalis and Cryptosporidium parvum are parasitic protozoans and infections are common in domestic ruminants, other wild and domestic animals and humans.
- Giardia duodenalis and Cryptosporidium parvum infections can lead to moderate to severe diarrhea with occasion mortalities. Clinical disease with Cryptosporidium parvum occurs in calves between 1 and 3 weeks of age lasting 1-2 weeks in duration. Giardiasis is observed in calves older than 3 weeks of age and is chronic.
- Giardia infection in ruminants causes intestinal malabsorption thereby reducing rate of body weight gain and affecting feed efficiency in growing animals by as much as 10%.
- Cryptosporidium andersoni colonizes the abomasum of up to 10% of older beef and dairy cattle. This organism has only recently been identified in feedlot calves and dairy cows. This parasite has been shown to infect cattle for several years.
- Cryptosporidium andersoni infections reduce rate of gain in finishing beef cattle and impair milk production in dairy cattle by inhibiting protein digestion.
- These diseases have tremendous ability to be transmitted from animal to animal and from animal to humans. These infections have led to a significant environmental and public health concern.
- Giardia infections can be treated with benzimidazoles (fenbendazole, albendazole), but there are currently no effective treatments for cryptosporidiosis. Clearly, effective methods need to be employed to treat, prevent or control these diseases due their effects on performance and public health significance.

Introduction

Giardiasis (giardiosis) is the infection caused by the flagellate intestinal protozoan, Giardia duodenalis, which is also known as Giardia lamblia or Giardia intestinalis. Giardia is a non-invasive parasite and it colonizes the mucosal surface of the small intestine. It exists as a trophozoite in the small intestine and an environmentally resistant cyst in the environment as shown in Figure 1. It is one of the most commonly identified intestinal pathogens in humans and animals throughout the world. There are some well-defined species: Giardia duodenalis (infects humans and most domestic and wild mammals), Giardia muris (infects rodents), Giardia psittaci (infects birds), Giardia ardeae (infects birds), and Giardia agilis (infects amphibians). These species are based upon morphology, biochemical characteristics, and host specificity. Presently the range of animals that each Giardia species may infect has not been clearly

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established. As shown in Table 1, molecular genotyping has been used to better characterize Giardia isolates. There are at least 3 genotypes of Giardia, which have been identified in cattle. One genotype appears to be restricted to hoofed animal species while others are common to other domestic and wild animal species (including humans). In Canada the predominant genotype (>80%) of cattle is the hoofed animal (O'Handley et al., 2000a). The hoofed animal genotype has never been reported in humans.

Cryptosporidiosis is also caused by another microscopic protozoan parasite, Cryptosporidium parvum. It exists in the gut cells as many different morphological forms and in the environment as a resist oocyst (Figure 1). It is associated with severe diarrhea in calves and humans. Beef and dairy cattle are infected with at least two distinct species (Cryptosporidium parvum and Cryptosporidium andersoni). Cryptosporidium parvum colonize the small intestine of calves while Cryptosporidium andersoni infect the abomasum of older cattle. As shown in Table 1, based upon molecular analysis and infection studies, there are numerous subspecies and genotypes of Cryptosporidium. For example, cattle strains of Cryptosporidium parvum are infective to humans; however, human strains are not infective to cattle.

Life Cycle and Transmission

The infection is obtained after the microscopic infective cysts (Giardia) or oocysts (Cryptosporidium) are ingested. These cysts and oocysts are resistant to the environment and remain infective for months in cold water or damp, cool environments. After the cysts/oocysts are passed through the stomach, they develop, multiply and colonize the entire small intestine. The cysts and oocysts, shown in Figure 1, have a protective carbohydrate wall that makes them resistant to environmental destruction. They are only destroyed by desiccation, heat, UV radiation and high concentrations of a few biocides. Table 2 demonstrates that cysts and oocysts can survive for months in water.

Ingestion of as few as 10 cysts/oocysts has led to infections in humans and animals (Wolfe, 1992). The most common method of transmission in animals and humans is the fecal-oral route (Xiao, 1994). Disease outbreaks have most often been attributed to the waterborne method of transmission (Wallis et al., 1996). It is believed that human effluent is the major source of water contamination, but certainly contamination of water with infected animal feces can lead to widespread infections in human and animals (Wallis et al., 1996). Water mammals, like beavers, have been implicated with many waterborne infections of Giardia. However, certainly other animals may be more likely candidates to contaminate water supplies. Foodborne infections have been associated with poor hygiene in food handlers and washing food with contaminated water (Wolfe, 1992). This is a particular concern as water is important in the processing of many foods.

Epidemiology

Giardia lamblia is one of the most commonly identified intestinal pathogens in humans and animals in the world (Wolfe, 1992; Xiao, 1994). It should be noted that prevalence rates are underestimated, because of the sensitivity of parasite detection and the intermittent nature of cyst excretion. In the past, humans, dogs, cats, and certain species of wildlife are described as the principal hosts of this parasite (Wolfe, 1992). Recent studies have identified domestic livestock (e.g., cattle, sheep, pigs, horses) as major hosts for this parasite (Olson et al., 1997a; 1997b; Xiao, 1994). Tables 3 and 4 describe the prevalence of Giardia and Cryptosporidium spp in Canadian farm animals. It should be noted that Giardia infections have been shown to be highly prevalent in all provinces of Canada and throughout the world (Olson et al., 1997a; 1997b). Cryptosporidium parvum is most commonly associated with intestinal disease in calves less than 4 weeks of age (Olson et al., 1997a; 1997b; O'Handley et al., 1999).

Infection in Cattle

In calves, Giardia and Cryptosporidium parvum infection patterns differ significantly as shown in Figure 3. Calves usually become infected with Cryptosporidium between 1 and 4 weeks of age and the duration of infection is short, lasting about 2 weeks (O'Handley et al., 1999). Calves become infected with Giardia between 4 and 10 weeks of age and infections persist for well over 120 days (O'Handley et al., 1999). Virtually all dairy calves develop cryptosporidiosis and giardiasis, but only some animals develop diarrhea. This is consistent with many intestinal infections of animals and man as disease is a complex interaction between the pathogen and the host. Cryptosporidiosis is less frequently observed in beef calves, but giardiasis was observed in 100% of animals (O'Handley et al., 1999; Ralston et al., 2001). Infected with Cryptosporidium in beef calves is usually more severe than infection in dairy calves. In Alberta and Saskatchewan, mortalities rates of up to 30% have been reported in beef calves while Cryptosporidium mortalities are infrequently observed in dairy calves (unpublished results). These cases of high Cryptosporidium mortalities are usually associated with the introduction of dairy calves to beef herds at the time of calving. Herds with high mortalities have also been consistently low in serum and tissue selenium suggesting that certain trace elements may also play a role in the immune status and clinical outcome of the calves.

Giardia causes disease by affecting the absorptive and digestive function of the small intestinal surface. It therefore induces a maldigestive and malabsorptive diarrhea. Cryptosporidium invades the small intestinal cells leading to cell and tissue destruction. In summary, clinical giardiasis and cryptosporidiosis are frequently not diagnosed, yet they may be the most common cause of diarrhea in calves on some farms.

Cryptosporidium andersoni infects calves greater than 3 months of age and this infection can persist for years. Abomasal Cryptosporidium invades the peptic glands causing dilation, hypertrophy of the gastric mucosa and thinning of the epithelial lining as demonstrated in Figure 3. Functionally this leads to impairment of protein digestion by increasing gastric pH and inhibition of proteolytic function of pepsin (Anderson et al., 1990).

Effect on Animal Performance

In humans and some animals, clinical signs of giardiasis and cryptosporidiosis include acute or chronic diarrhea, abdominal pain, dehydration, weight loss, and reduction in weight gain. The most common clinical sign, although often not recognized, is weight loss and reduced weight gain. Giardiasis has been associated with growth retardation and disease susceptibility in children from endemic areas (Wolfe, 1992). There have been reports of clinical signs in cattle and sheep, which include diarrhea and weight loss (Olson et al., 1995). Most Giardia infections in ruminants have been described as asymptomatic (Xiao, 1994). In growing ruminants giardiasis has a negative effect on production leading to a reduced rate of gain without a reduction in feed intake suggesting malabsorptive and/or maldigestive disease. Infected animals may also have lower carcass weight and an increase time to slaughter (Olson et al., 1995). Table 1 shows that experimentally infected Specific Pathogen Free lambs had a decrease in body weight gain and feed efficiency. We have recently demonstrated that the intestinal pathology of calves with Giardia rapidly recovers following treatment with fenbendazole which is demonstrated in Figure 4 (O'Handley et al., 2001). Chemotherapeutic agents such as fenbendazole may be useful in improving performance in Giardia infected calves (O'Handley et al., 2001).

Cryptosporidium parvum often causes severe watery diarrhea in calves less than 3 weeks of age (O'Handley et al., 1999). It is believed to have a significant impact on the growth of calves (Olson et al., 1997a). Some animal may die if appropriate supportive medical attention is not provided. These calves

usually do not gain weight and frequently loose weight during the infection but they usually recover uneventfully after they have cleared the parasite (about 2 weeks). There is no information on the long-term effects of Cryptosporidium infection in calves. Older calves infrequently have clinical signs of cryptosporidiosis.

Cryptosporidium andersoni infections can cause moderate to severe impairment of weight gain and decreased feed efficiency in feedlot cattle (Anderson et al., 1990; Esteban and Anderson, 1995). We have identified up to 10% of feedlot cattle with chronic C. andersoni infections (Ralston et al., 2001). These cattle are frequently late to finish compared to their penmates. There can be a reduction in performance of up to 50% in feedlot cattle (Anderson, 1990; Esteban and Anderson, 1995; Ralston et al., 2001). In one study 9% of diary cattle were infected with C. andersoni and infected cattle produced significantly less milk (approximately 3.2 kg/day) (Esteban and Anderson, 1995). The effects of C. andersoni on range cattle is unknown but the known nutritional and milk production effects of this abomasal parasite would be expected to affect growth in nursing range calves.

Animal to Human Transmission (Zoonoses)

Giardiasis is considered to be an important zoonotic disease and animal reservoirs are believed to be cats, dogs, ruminants, and a variety of wild animals. Infections in domestic ruminants are of special concern because of the potential contamination of surface and ground waters through pasture run-offs and the distribution of manure on fields. Outbreaks from water-borne giardiasis and cryptospordiosis in humans have been attributed to pasture run-off leading to drinking water contamination. Morphological, protein, and DNA similarities among Giardia isolated from animals and humans have been demonstrated. The number of clinical reports and transmission studies suggest the possible zoonotic transmission of the Giardia parasite (Wolfe, 1992).

Similarly, Cryptosporidium parvum from cattle have been clearly demonstrated to be transmissible to humans, but isolates from other animals may not be infective to humans (Sulaiman et al., 1998). Cryptosporidium infections are infrequent, but waterborne outbreaks can lead to large numbers of human infections as occurred in Milwaukee where 400,000 humans were believed to have contacted the disease (Wallis et al., 1996; Sulaiman et al., 1998). We have demonstrated that 60% of humans with clinical Cryptosporidiosis in the Calgary Health region to be infected with the bovine genotype (unpublished results). This infection may or may not have resulted from directly associated with cattle or water contaminated by cattle as human-to-human transmission of the bovine genotype can occur. However, this data clearly indicates that bovine genotypes are a significant health risk to human populations.

Diagnosis

Giardia and Cryptosporidium are diagnosed by examination of fresh or formalin fixed fecal samples. Cyst and oocysts are concentrated using zinc sulphate or sucrose gradients (SG 1.18) to enhance the diagnosis. As illustrated in Figure 1, cysts and oocysts are best demonstrated using fluorescent monoclonal antibody (Olson et al., 1997a; 1997b). Giardia cysts can be stained with Lugol□s iodine and Cryptosporidium oocysts with a Ziel-Nelson stain. Diagnosis requires high quality microscopic equipment and training in recognizing these parasites. Recently fecal immunoassays have become available but the tests are expensive and are not used for routine testing by most laboratories. Examination for Giardia and Cryptosporidium are infrequently performed under routine microbiological and parasitological examinations in cattle. In addition, the sensitive assays described above may not be familiar to the diagnostic laboratory and most veterinary clinics do not have the equipment or expertise to reliably diagnose these parasitic diseases. For these reasons giardiasis and cryptosporidiosis are largely under-diagnosed in cattle.

Treatment and Control

There is no current approved treatment for giardiasis and cryptosporidiosis in cattle. There are a limited number of drugs available for the treatment of giardiasis in domestic animals. Metronidazole, tinidazole, quinacrine and furazolidone are effective, but they should be avoided in food animals because they are carcinogenic and mutagenic. Benzimidazoles (fenbendazole, mebendazole, albendazole) have recently been shown to be effective in elimination of Giardia lamblia in cattle (O'Handley et al., 1997). Fenbendazole at doses of 5 mg/kg daily for 3 days or 0.7 mg/kg daily for 6 days is highly effective in controlling ruminant giardiasis (O'Handley et al., 1997). Figure 4 clearly shows that the intestine of calves rapidly recovers following treatment with fenbendazole and intestinal lactase and maltase activity similarly responds to treatment (O'Handley et al., 2001). Indeed, there may be an economic benefit to treat infected calves from enhanced performance. Calves may need to be retreated as re-infection occurs rapidly after drug has been withdrawn (O'Handley et al., 2000b). A Giardia vaccine (GiardiaVax, Fort Dodge Animal Health) is commercially available for dogs and cats but has not been evaluated in cattle (Olson et al., 2000). Vaccination may be an option for controlling Giardia in calves.

Cryptosporidiosis is frequently a self-limiting disease in young calves lasting for 2 weeks. The severity of clinical disease may be associated with the animals' immune and nutritional status. Recently problem herds have been shown to be deficient in selenium (Olson, unpublished results). There is currently no satisfactory chemotherapeutic agent available for the treatment of cryptosporidiosis. Many agents have been tested in vitro and in vivo, but few agents have shown promise. Drugs such as paromomycin, halfuginone, and lasalocid have shown promise in calves. Paramomycin is expensive and halfuginone and lasalocid are highly toxic at effective doses. Cryptosporidiosis in calves should be treated with fluid therapy and correction of acid-base disturbances. Antibiotics can be used to control secondary bacterial infections. Colostrum, which contains anti-Cryptosporidium antibodies, may also be beneficial. Experimental vaccines have been produced and this vaccine may provide an optimal method for controlling cryptosporidiosis in calves.

Currently there have no studies that have investigated the treatment of Cryptosporidium andersoni in the abomasum of feedlot or dairy cattle. Certainly, the impact of this infection on performance and production would warrant such studies.

Implications

Giardia duodenalis, Cryptosporidium parvum and Cryptosporidium andersoni are gastrointestinal protozoan parasites of cattle that have gone largely unrecognised until very recently. These parasites can cause clinical diseases but they also can affect performance in young calves and feedlot cattle. An economic benefit should be achieved by controlling these infections by means of management, chemotherapeutics and vaccination.

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Table 1. Species, Genotypes and Host Specificity of Giardia and Cryptosporidium

Species	Genotype	Host Range
Giardia duodenalis	Assemblage A	humans, cattle, pigs, horses, cats, dogs, beavers, guinea pigs, non-human primates
	Assemblage B	humans, chinchillas, dogs, beavers, rats non-human primates
	Dog	dogs
	Cat	cats
	Livestock	cattle, goats, sheep, pigs, alpaca
	Rat	rats
	Muskrat	muskrats, voles
Giardia microti		muskrats
Giardia muris		mice
Giardia agilis		amphibians
Giardia psittaci		birds
Giardia ardeae		birds
Cryptosporidium parvum	Cattle	cattle, humans
	Human	humans
	Mouse	mouse
	Pig	pigs
	Marsupial	marsupial
	Dog	dogs, humans
	Ferret	ferrets
Cryptosporidium muris		rodents (stomach)
Cryptosporidium andersoni		cattle
Cryptosporidium felis		cats, humans
Cryposporoidium wrairi		guinea pigs
Cryptosporidium meleagridis		turkeys, humans
Cryptosporidium baileyi		chickens
Cryptosporidium serpintis		snakes
Cryptosporidium nasorum		fish

Table 2. Survival of Animal Fecal Pathogens in the Environment

Material	Temp	Duration of Survival					
		Giardia	Crypto-sporidium	Salmonella	Campylo-bacter	Yersinia entercoliticia	E. coli O157:H7
Water	frozen	< 1 day	> 1 year	>6 mos	2-8 wks	> 1yr	>300 days
	cold (5C)	11 wks	> 1 year	>6 mos	12 days	>1 yr	>300 days
	warm (30C)	2 wks	10 wks	>6 mos	4 days	10 days	84 days
Soil	frozen	< 1 day	> 1 yr	>12 wks	2-8 wks	>1yr	>300 days
	cold (5C)	7 wks	8 wks	12 - 28 wks	2 wks	> 1yr	100 days
	warm (30C)	2 wks	4 wks	4 wks	1 wk	10 days	2 days
Feces	Frozen	< 1 day	> 1 yr	>6 mos	2-8 wks	>1yr	>100 days
	Cold (5C)	1 wk	8 wks	12-28wks	1-3 wk	30-100 days	>100 days
	Warm (30C)	1 wk	4 wks	4 weeks	1 wk	10-30 days	10 days
Slurry		1 yr	>1 yr	13-75 days	>112 day	12-28 days	10-100 days
Compost		2 wks	4 wks	7- 14 days	7 days	7 days	7 days
Dry Surfaces		1 day	1 day	1- 7 days	1 day	1 day	1 day

Table 3. Prevalence of Giardia and Cryptosporidium in Canadian Farm Livestock

Animal	No. Sampled	Giardia No. Positive (% Positive)	Cryptosporidium No. Positive (% Positive)
Cattle	104	30 (29)	21 (20)
Sheep	89	34 (38)	21 (24)
Pigs	21	9 (9)	9 (11)
Horses	35	7 (20)	6 (17)

Sample locations: Vancouver, British Columbia; Strathmore, Alberta; Swift Current, Saskatchewan; Brandon, Manitoba; Lennoxville, Quebec; Fredericton, New Brunswick; Airdrie, Alberta; Saskatoon, Saskatchewan; Nappan, Nova Scotia; Truro, Nova Scotia; Whitehorse, Yukon; Calgary, Alberta; Edmonton, Alberta; Guelph, Ontario; Innisville, Alberta.

Table 4. Prevalence of Giardia spp and Cryptosporidium spp in Alberta and British Columbia Dairy and Beef Calves

	Location	Number	% with Diarrhea	Giardia	Crypto parvum	Crypto andersoni
Beef Calves	BC	192	30%	37%	11%	0%
Range Cattle	BC	33	-	36%	6%	33%
Diary Calves	BC	386	7%	73%	15%	2%
Beef Calves*	Alberta	20	-	100%	5%	0%
Dairy Calves*	Alberta	20	56%	100%	100%	0%
Feed lot Cattle	Alberta	60	-	82%	25%	82%
Range Cattle	Alberta	280	-	32%	9%	18%

* Longitudinal study following calves from birth until 120 days of age

Table 5. Influence of Giardiasis on Production in an Experimental Lamb Infection Model

Variable	Control*	Infected*	P Value
Birth Weight	3.96 + 0.14	3.96 + 0.14	0.987
Weaning Weight (kg)	15.22 + 0.35	15.13 + 0.46	0.882
Week 5 Weight (kg)	20.23 + 0.51	20.11 +57	0.884
Week 16 weight (kg)	44.00 + 1.03	41.96 + 1.08	0.177
Weight gain (wk 5-16, kg)	28.78 + 1.40	26.82 + 0.86	0.107
Feed Intake (wk 5-16, kg	99.1 + 3.8	101.0 + 4.0	0.346
Feed Efficiency (wk 5-16)	3.44 + 0.11	3.77 + 0.11	0.041
Days to slaughter	121.9 + 1.7	124.0 + 1.6	0.270
Final Live weight (kg)	45.73 + 0.86	44.28 + 0.93	0.260
Carcass Weight (kg)	21.91 + 0.48	20.60 + 0.41	0.046

* Mean + SEM

Figure 1. Giardia duodenalis cysts and Cryptosporidium oocysts stained with a fluorescent antibody.

Figure 2. Course of infection of Giardia and Cryptosporidium in Calves

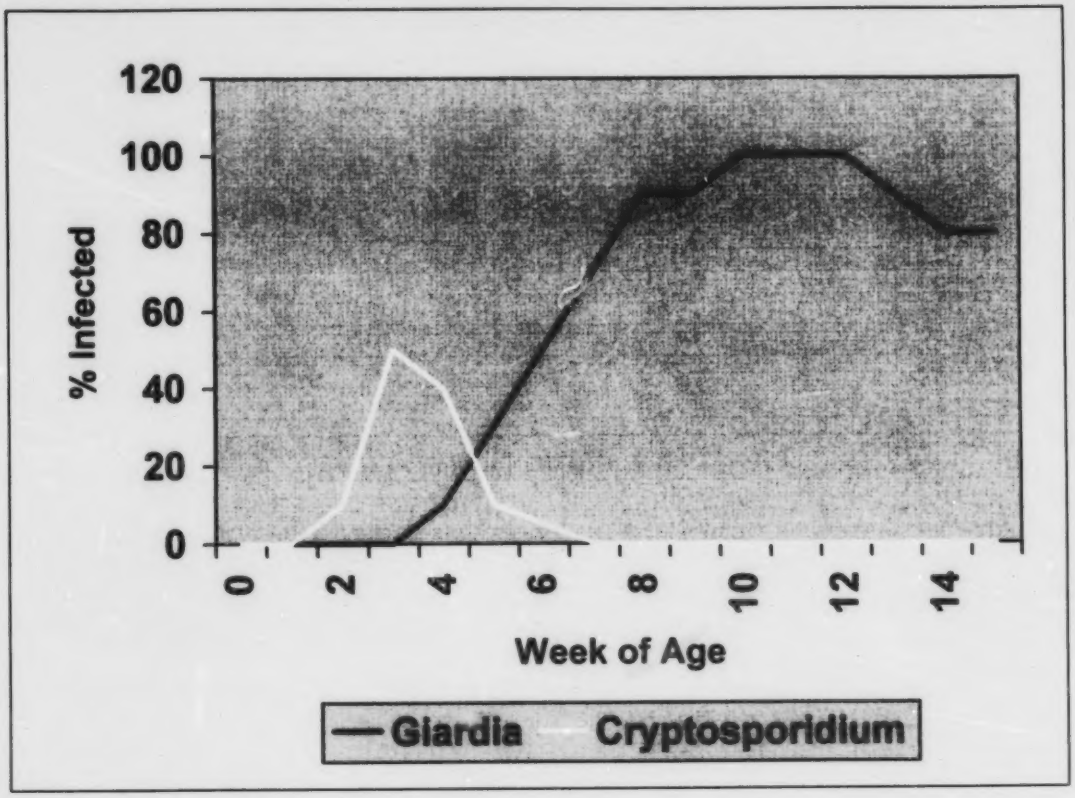
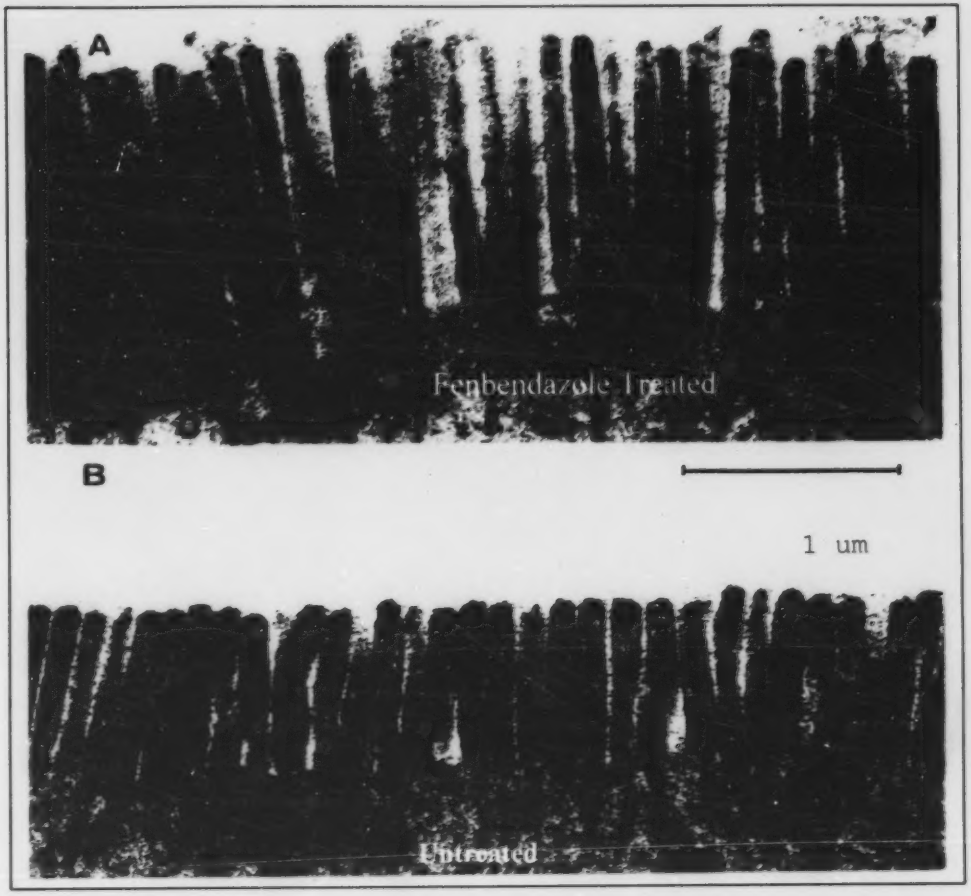


Figure 3. Cryptosporidium andersoni infection in the abomasum of a feedlot steer. Note how the Crypotosporidium morphotypes are dilating the gastric crypts (arrows).

Figure 4. Appears of Intestinal Microvillus 7 Days following trreatment for Giardia



The Canada Alberta Beef Industry Development Fund (CABIDF)

About Us

Fund structure. The Canada Alberta Beef Industry Development Fund (CABIDF) is a $16.4 million fund established with equal contributions from the Federal and Alberta governments. The Fund is available because Alberta producers chose to allocate a portion of their safety net money to market-neutral industry development.

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Fund partners are the Agriculture and Agri-Food Canada (AAFC) and Alberta Agriculture, Food and Rural Development (AFFRD). The Alberta Cattle Commission (ACC), representing all cattle producers in the province, oversees Fund administration. The research funding selection process is overseen by the Alberta Agricultural Research Institute (AARI).

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Fund activities are overseen by a board of directors. Voting members are a core group of five producer representatives selected to directly represent producer interests: Chair, Larry Helland, cow-calf operator and past chair of the Alberta Cattle Commission; cattle feeders Ed Thiessen and John Prentice; rancher and seedstock producer Neil Harvie; and Bob Nelson, former forage specialist for AAFRD. Non-voting advisors on the Board include: Dr. Steve Morgan Jones, long-time meat researcher, now Director of AAFC's Lethbridge Research Centre; Dale Engstrom, Manager of the Beef and Dairy Group of AAFRD, and Don Milligan of the Alberta Agriculture Research Institute.

Assessing the Risk of Bluetongue Transmission in Canada

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Key Points

- Bluetongue is a viral disease that affects cattle movement and trade.
- A small midge, Culicoides sonorensis, is the biological vector of bluetongue.
- The vector is present in western Canada, but the limits of its distribution are unknown.
- The ability of vector populations to transmit the virus varies geographically, and is influenced by a variety of factors.
- We are currently conducting studies of vector ecology to determine the potential for transmission of bluetongue under Canadian conditions following importation of infected cattle.

Introduction

Bluetongue virus is vectored by various species of biting midges in the genus Culicoides. Bluetongue causes a debilitating and often fatal disease in sheep and some species of wild ruminants including bighorn sheep, deer, and antelope. The disease originated in Africa (Moulton, 1961), but has become distributed almost worldwide between 35°S and 40°N (Mellor et al., 2000). An outbreak in Spain caused the death of 179,000 sheep (75% mortality of those affected) during 1956-1960. In affected animals, bluetongue causes fever, swelling of mouth and nasal passages, salivation, swollen tongue, hemorrhages, and oral erosion. Vomiting may occur and cause pneumonia (Moulton, 1961). Mortality can be substantial. Outbreaks in wildlife not only cause large-scale die-offs, but also reduce reproduction in the longer term (Thorne et al., 1988). The disease is largely asymptomatic in cattle, but prenatal infection may result in the death of the fetus. Cattle will develop a viremia that can last for long periods and act primarily as a reservoir and amplifying host (Barratt-Boyes and MacLachlan, 1995).

Bluetongue is widely distributed in the United States, but is most prevalent in southern and western states where the incidence can reach 79% based on serological survey. The disease is rare or absent in the northeastern States, and also uncommon in northern States such as Montana, Idaho, Washington, and North Dakota (Metcalf et al., 1981). This pattern has remained stable for a number of years (Pearson et al., 1992).

Bluetongue was detected in cattle in the Okanagan Valley of British Columbia in 1975 during routine serological testing of imported cattle. Antibodies were also found in 221 native Canada cattle indicating that local transmission occurred (Dulac et al., 1992). This resulted in curtailed exports of cattle and semen (Sterritt and Dulac, 1992). Bluetongue has been further detected in the Okanagan Valley in 1987, 1988 (Dulac et al., 1992; Sterritt and Dulac, 1992) and 1998 (CFIA, 1998). With the

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exception of the Okanagan Valley, Canada is considered bluetongue-free and maintains this status by restricting import of feeding cattle from the United States during the summer. In addition to being considered a serious trade irritant, these import restrictions cost both Canadian and American cattle producers in terms of testing, increased cost of cattle purchase, and underutilization of feedlot space. Canadian producers would benefit greatly by importing feeder cattle year round.

Although considerable economic benefit could be gained by allowing year round imports into Canada, it is still unclear whether introduction of cattle infected with bluetongue virus could result in the transmission of the bluetongue virus to cattle, sheep and wildlife populations. The vector is the critical element in the transmission of bluetongue, and many believe that Canada lacks a competent vector. The purpose of this review is to discuss the biology of the bluetongue vector in Canada, and its potential to transmit the virus in following potential introduction.

Vector Identification

Bluetongue virus is vectored by small biting midges in the genus Culicoides. These midges belong to the family Ceratopogonidae, and are also called no-see-ums, biting midges, punkies and sand flies. The adults are about 1-3 mm long. Females have piercing and sucking mouthparts that are directed downwards and are as long as the head. Palps protrude from the mouthparts and are slightly longer than the mouthparts. The 15-segemented antennae are plumose in males while the females have beaded antennae. The wings have a characteristic rounded shape, no scales, and are folded scissor-like over the abdomen at rest (Downes and Wirth, 1981).

Over 1400 species of Culicoides have been described worldwide (Mellor et al., 2000) but relatively few can transmit bluetongue. Vector species vary from region to region around the world. In North America, the principal vector is now recognized as Culicoides sonorensis (Tabachnick, 1996; Holbrook et al., 2000). C. sonorensis was originally considered one of 5 subspecies of the Culicoides variipennis complex that included C. variipennis variipennis, C. v. occidentalis, C. v. sonorensis, C. v. australis, and C. v. albertensis (Wirth and Jones, 1957). These are now considered three separate species, C. variipennis, C. occidentalis, and C. sonorensis. Culicoides sonorensis includes the three previous subspecies C. v. sonorensis, C. v. australis, and C. v. albertensis (Holbrook et al., 2000).

The three species are difficult to identify as they are quite small and show considerable geographic variation in many characters. All have similar patterns of spots on the wings that distinguish them from other species of Culicoides, but not among themselves. Females of C. sonorensis and C. occidentalis cannot be distinguished morphologically but are distinct from C. variipennis. Female C. sonorensis and C. occidentalis have a swollen third palpal segment bearing a large sensory pit while female Culicoides variipennis have a more slender third palpal segment. Male C. sonorensis are distinct, having a spiculate aedeagus in comparison to the smooth aedeagus of the other two species (Holbrook et al., 2000). The species can be resolved using genetic analysis (Tabachnick, 1992).

Vector Distribution in Western Canada

A great deal of confusion has surrounded the geographic distribution of the vector, particularly in relation to the distribution of bluetongue. Much of this confusion has arisen because of the difficulty in identifying the species. Since bluetongue is rare in the northern United States and Canada outside of the Okanagan Valley, it is often assumed that a competent vector is absent in these areas. This is only partially true. C. variipennis is the sole species in the northeastern United States where bluetongue is absent (Tabachnick, 1992) and it is not a competent vector (Tabachnick, 1996),

which does explain the lack of bluetongue in that area. C. sonorensis is a competent vector, and is the species most abundant in areas where bluetongue is endemic (Tabachnick, 1996). However, bluetongue has never been detected in western Canada outside of the Okanagan Valley, and this is sometimes viewed as evidence that western Canada lacks the vector species.

Even though bluetongue is absent in western Canada, C. sonorensis is present in western Canada. There are several lines of evidence for this. The description of C. v. albertensis was based on specimens collected at Lethbridge, Fort MacLeod, and Brooks, AB in 1955, and C. v. albertensis is now considered C. sonorensis (Holbrook et al., 2000). Genetic analysis of Culicoides collected near Warner, AB indicated C. sonorensis (Holbrook et al., 2000). Additionally, Holbrook et al. (2000) examined topotypes of C. v. albertensis from Southern Alberta, Canada, and confirmed they were typical C. sonorensis. Finally, Mr. Joe Shemanchuk of our laboratory collected C. sonorensis at 7 locations in southern Alberta in 1988, and we have confirmed they are C. sonorensis based on the spiculate aedeagus. While it is clear that the vector is present in Alberta, it still remains to determine how far north and east C. sonorensis occurs, and whether or not it is present in Ontario.

Vector Life Cycle and Biology

The life cycle of Culicoides sonorensis has 4 stages: egg, larva, pupa, and adult. Eggs are banana shaped, and are laid in moist soil near the edges of standing water. The eggs hatch in 2 –3 d, and the larvae develop in sediment in near the edges of ponds with gently sloping shorelines (Mullens, 1989). C. sonorensis larvae are particularly abundant in waters polluted with livestock waste (Schmidtmann et al., 2000).

The larvae are vermiform, with a sclerotized head, 3-thoracic and 9-abdominal segments, and lack spiracles. Larvae move with a characteristic wiggling motion. Larval developmental time requires about 2 – 3 weeks but varies with temperature and nutrition (Vaughan and Turner, 1987; Mullens and Velten, 1994). The larvae pass through 4 instars, each progressively larger, and pupate in moist soil on shore. Pupae are compact with a short prothorax bearing a dorsal respiratory horn. Pupae complete development in 2 –3 days.

Adults emerge and will mate after a day. Although most species of Culicoides mate in large swarms, C. sonorensis will mate on the host (Gerry and Mullens, 1998) and in confined areas (Downes, 1978), making establishment of laboratory colonies possible. Adult females feed on vertebrate blood, and are attracted by carbon dioxide (Mullens and Gerry, 1998). The host range is broad and adults seem to be especially attracted to cattle. Adults are poor fliers, and generally occur near larval habitat. Only female midges feed on blood, but both the females and males will feed on vegetable fluids and nectar. Flight does not occur when wind is faster than 7 km/h and they primarily bite during dawn and dusk (crepuscular). Blood is required for the females to develop eggs.

Blood feeding is cyclical and timed with ovarian development. Females will take a blood meal, develop a batch of eggs, lay eggs, and then take another blood meal. The time to develop eggs following a blood meal is highly dependent on temperature, ranging from 2 d at 34°C to 13 d at 13°C (Mullens and Holbrook, 1991). These authors have developed an equation for the length of egg development, but it is unclear if the time required to develop a second batch of eggs is less than the time required to develop the first, as has been noted for other blood-feeding flies (Lysyk and Krafsur, 1993). Females lay about 60 – 70 eggs per batch (Mullens and Holbrook, 1991). Adult survival is highly dependent on temperature (Hunt et al., 1989; Gerry and Mullens, 2000). Adults appear in May in Alberta and population peaks occur at approximately 1-month intervals. These probably reflect new generations emerging from larval developmental sites. Adults are scarce after mid-September, and the species overwinters as slowly developing larvae in unfrozen pond sediment.

Virus Transmission

The midge acquires the virus by feeding on the blood of an infected animal. The virus replicates within the midgut cells, then moves into the hemocoel, and is distributed to other tissues via the insect's blood. The virus infects the salivary glands, replicates, and can be transmitted to a new host during feeding only after the virus replicates in the salivary glands (Ballinger et al., 1987). The time between when the virus is acquired by the vector and can be transmitted by the vector is termed the extrinsic incubation period and its duration is highly influenced by temperature (Mullens et al., 1995). The extrinsic incubation period ranges from 4 d at 32°C to 14 d at 21°C (Gerry and Mullens, 2000). The midges may take several blood meals between the time the midge is first infected and the time the midge can transmit the virus.

The virus does not cross the ovaries of the females (Ballinger et al., 1987). As a result, there is no vertical transmission of the virus from an infected midge to her own progeny. The overwintering mechanism of the virus therefore remains somewhat of a mystery. The overwintering reservoir is mostly likely infected animals with a long viremia.

Factors Influencing Transmission

A variety of factors influence the transmission of a disease agent by arthropods. These include (Gerry and Mullens, 2000):

1. Vector competence, the genetically determined susceptibility of the vector to infection and its permissiveness for pathogen development;
2. Biting intensity, the number of bites per host per unit time which is determined by vector population size and environmental factors;
3. Vector survival;
4. The length of time between blood feedings which is determined by reproductive development and temperature;
5. The extrinsic incubation period of the virus.

Vector competence will determine the proportion of midges feeding on a viremic host that can become infected. This varies both among and within species. It is reasonably well established that C. sonorensis is the principal vector of bluetongue in North America (Tabachnick, 1996), but populations vary in their susceptibility to infection by the virus. Nunamaker et al. (1997) used 10 – 30% as estimates of vector infection. Biting intensity has not been determined in Canada, but approximately 70 – 110 bites/host/d were associated with bluetongue transmission in California (Gerry et al., 2001). Vector survival, the length of time between blood feedings, and the extrinsic incubation period of the virus are determined by temperature (Gerry and Mullens, 2000; Gerry et al., 2001) and vary greatly within a season and among locations (Work et al., 1991). It is likely that an infected midge must survive for a total of four blood meals transmit the virus (Mullens and Rutz, 1984), as the extrinsic incubation period of the virus is greater than the time between blood meals. Nunamaker et al. (1997) suggested that midge survival has the greatest impact on the potential for transmission.

We have developed a collaborative, multi-disciplinary study with participation from the Canadian Cattle Industry, Agriculture and Agri-Food Canada, and USDA to examine the potential for transmission of bluetongue virus in Canada under our environmental conditions and various cattle importation scenarios. We will be conducting studies of vector ecology in Alberta, examining virus development in Canadian strains of C. sonorensis, and determining the prevalence of infection in cattle entering Canada. This information will be integrated into a model that will allow us to

determine the proportion of a vector population that could survive long enough to transmit the virus in various regions of Canada.

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Session III - Environmental Issues

Speakers:

Dr. Steve Hrudey (no manuscript submitted)
Dr. Henry Janzen
Dr. Helen Fairweather

Reducing Greenhouse Gas Emissions from Agriculture: Challenges and Opportunities

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Key Points

- Despite lingering uncertainties, climate change is now widely viewed as a serious problem, and agriculture will be asked to help reduce emissions of greenhouse gases.

- Greenhouse gas emissions point us to inefficiencies in our farming systems; correcting these inefficiencies can sometimes increase productivity, lower costs, reduce other environmental effects, and – as a very attractive bonus – reduce emissions of greenhouse gases.

What is the problem?

In recent years, our newspapers and airwaves have suddenly been inundated with headlines about the 'greenhouse effect'. But the phenomenon is not new – it has been around since life on this planet began. Greenhouse gases, such as carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and water vapor (H_2O), trap radiation going outward from the earth into space, warming the atmosphere near the earth's surface. Without this effect, the earth's average temperature would be about 33° C lower than it is now, and our planet would be cold and barren.

If the 'greenhouse effect' is a good thing, then what is the worry? The problem is that concentrations of greenhouse gases in the atmosphere are increasing, largely from human activities: CO_2 concentration has increased by 31% since the industrial revolution, N_2O by 16%, and methane by about 150% (Table 1). And the increases continue; CO_2 concentrations, for example, will likely reach values double the pre-industrial levels before this century ends. If the concentrations of greenhouse gases – the gases that generate the envelope of warmth – are increasing, can we reasonably expect that climate will be unaffected?

The Intergovernmental Panel on Climate Change (IPCC) now projects that the global temperature will increase, from 1990 to 2100, by 1.4 to 5.8° C (IPCC, 2001). A temperature change at the top end of this range is not unlike that from an ice age to a subsequent warm period (Houghton, 1997). But temperature change is not the only threat; there are other worries: Will precipitation be affected and will severe weather events occur more often? Will sea levels rise or human disease be more widespread or food become more scarce? We cannot predict temperature change precisely; we know even less about the other ill effects. But there is now growing consensus that changes will happen, if they have not already. And though we cannot predict with certainty what those changes will be – some might even be favorable – the uncertainty itself is a worry; impending changes we cannot predict may be more disconcerting than ones we can.

Prompted by building fears of climate change, the world's nations have started looking for ways of reducing emissions of greenhouse gases. In 1997, they reached agreement on the Kyoto Accord. Its targets are quite modest; if reached, they would reduce emissions from participating countries in the years 2008-2012 by about 5% below 1990 levels. Even then, meeting the Kyoto targets is not a small undertaking. Canada, for example, has agreed to reduce emissions by 6%, relative to 1990; but emissions are already well above 1990 levels – and climbing – so actual cuts in

emissions, relative to business-as-usual, may be about 25%, rather than 6%. Can we find painless ways of cutting energy use and other emissions by a quarter?

Where does agriculture fit?

Agriculture emits a lot of greenhouse gases. Globally, emissions of CO_2, N_2O, and CH_4 from agriculture account for about 20% of the annual increase in radioactive forcing (Cole, 1996). Aside from its direct emissions, agriculture may result in CO_2 emissions when land is first cultivated for crop production. Land use changes such as deforestation, some of which are for agriculture, account for another 14% of global greenhouse gas emissions. In industrialized countries, agriculture accounts for a smaller proportion of greenhouse gas emissions. In Canada, it accounts for about 10% of emissions from human activities.

What are the sources of these emissions on Canadian farms? The largest source – accounting for about 60% – is the N_2O released from agricultural soils. Nitrogen added to soil in fertilizers, manures, and other amendments, as well as the N already present in soil, is changed in form by soil microbes. For example, ammonium forms are changed to nitrate (called 'nitrification') or nitrate is converted to N_2 gas ('denitrification'). During these processes, a small amount of the nitrogen may be released as N_2O. Highest amounts are usually released shortly after nitrogen additions, or during periods when the soil is very wet. In many farmlands of Canada, the largest emissions occur immediately after snow melt, when the soil is wet. And when N is lost from soils, by leaching or to the air, it may eventually lead to N_2O emissions from groundwater or wherever else it is deposited.

Most of the remaining greenhouse gas emissions from farms occur as CH_4. Methane (CH_4) is produced when organic material decomposes under conditions of low oxygen supply. This happens in flooded soils (e.g., rice paddies) and in manure storage facilities, especially when high moisture content or liquids reduce aeration. And it occurs in the rumen of livestock, where feed is pre-digested under conditions of low oxygen supply. In general, the longer the feed remains in the rumen, the more CH_4 is released from the animals. In Canadian agriculture, most of the CH_4 comes from the rumen of cattle (Table 2).

At one time, farmlands released large amounts of CO_2 from decomposing organic matter – about 30% of the organic carbon once stored in the top layer of these soils was lost – but these CO_2 emissions have now largely abated (Janzen et al., 1998). And with improved management, it may be possible to recover a part of the carbon lost, thereby removing some CO_2 from the air (carbon sequestration).

Now that there is growing pressure to reduce emissions, what can we in agriculture do? Conceptually, there are three broad options:

1. We can find ways of reducing emissions: Most of agriculture's emissions occur as N_2O and CH_4. By better managing carbon and nitrogen flows in agroecosystems, we can reduce the amount of N and C escaping as these gases. As well, we can look for ways of using energy more efficiently, thereby stemming emissions of CO_2.

2. We can generate alternative fuels: By growing crops dedicated to making fuel (e.g., ethanol from corn) or trapping methane from agricultural by-products (e.g., from stored manure), we can reduce the amount of fossil fuels used. Such bio-fuels still generate CO_2 when burned, but they do not inject 'new' CO_2 into the atmosphere, merely re-cycling the atmospheric CO_2 recently fixed by photosynthesis. (Of course, this option is effective only if it does not rely unduly on fossil fuel to generate the biofuel.)

3. We can store excess CO_2 in soils: Agricultural soils contain large reserves of C in organic matter; a grassland soil, for example, may contain 100 Mg C ha^{-1} in the surface 30 cm. The soil C is linked to atmospheric CO_2: photosynthesis traps CO_2 in plant tissues, which become soil organic matter, and organic matter already in the soil is always decomposing back tc CO_2. Any practice that increases the amount of plant litter entering soil or slows the rate at which it decomposes in the soil will build soil C, removing CO_2 from the atmosphere.

Within each of these broad categories are a wide range of specific practices, a few of which are listed in Table 3. Practices that may reduce emissions from cattle operations include: improving rations (e.g., using high-grain diets in feedlots), using supplements or vaccines that suppress CH_4 production (e.g., ionophores), genetically altering animals or the microflora within them, and managing manures to improve aeration or otherwise slow rates of CH_4 formation. Further, the rangelands and forages used for feeding animals may also help reduce net emissions, if they can be managed to store more carbon in soil. Some of these examples have been widely tested and shown to be reliable; others are still in early stages of research. And more options may become available with time; as incentives to limit emissions intensify, new ways will be proposed, swelling further the list of potential mitigation practices to choose from.

How do we choose the best ways of reducing emissions?

Given the long and growing list of practices that may reduce emissions, how do we choose the best methods? How do researchers and producers and policy-makers decide on the best set of practices to adopt on the farm? Recommending the best practices is beyond the reach of this paper – our understanding is still incomplete, and the practices to be recommended will vary from region to region, even from farm to farm, depending on land, climate, economics, and social factors. Instead, I offer for debate a few observations that may serve as guiding principles in choosing how best to reduce emissions from our farms and our industry.

1. Look at the whole system

When looking at possible ways to reduce emissions, we often look only at one part of the system. For example, soil scientists will ask: What practice sequesters the most carbon in the least time? And animal nutritionists may ask: What feed formulation gives the lowest CH_4 emissions? And an engineer may ask: What method of storing manure releases the least CH_4? But choosing a practice on one part of the farm may alter emissions elsewhere – a new ration that reduces CH_4 emission from beef cattle may generate more N_2O during the production of that feed; a cropping system that stores more soil carbon may generate more CH_4 or CO_2 when the products are used downstream. Blindly advocating a practice because it is effective in one part of the system may lead to surprises, good or bad, elsewhere.

Suppose, for example, that a farmer decides to introduce a perennial grass/legume forage into a crop rotation that before only had annual crops (Fig. 1). This change results in increased soil carbon content, thereby reducing net CO_2 emissions. But when the forages are fed to ruminant livestock, they lead to increased CH_4 emissions. And manure produced by the livestock results in further emissions of CH_4 and N_2O. But the legumes fix nitrogen and the manure re-cycles nitrogen onto the land so that less nitrogen fertilizer is needed, reducing N_2O emission and also cutting CO_2 release (making nitrogen fertilizer is very energy intensive – about 4 kg CO_2 are released per kg of N). Thus one management change has influenced all three greenhouse gases. And this example still does not yet include all the indirect effects on greenhouse gas emissions.

Clearly, if the intent is to reduce net greenhouse gas emissions, then it is not enough to know how much carbon is sequestered by a new tillage practice, or how much N_2O is suppressed by a new fertilizer practice, or how much CH_4 is not emitted with a new feeding program. We need to consider (and first understand) how a proposed practice affects emissions of all gases from all parts of the farming system.

2. Focus on reducing emissions per unit of product

Agricultural ecosystems serve many functions – wildlife habitat, recreation, environmental filter, and, perhaps foremost, producing food. Consequently, if we want to evaluate how effectively a practice reduces emissions, we need to consider how that practice affects performance. For example, if a key function of the ecosystem is to produce food, then a mitigation practice may be best evaluated by measuring how it affects emissions per unit of product (e.g., emissions per tonne of protein).

From this perspective, one way of reducing emissions is to increase production. For example, a new feeding method could increase CH_4 emissions per animal, but reduce the amount of CH_4 emitted per kg of beef sold. A new fertilization method may increase N_2O emitted per ha, but reduce N_2O emissions per tonne of wheat protein. Increased product yield, however, will reduce overall greenhouse gas emissions only if the higher productivity at one site negates production elsewhere.

One view suggests that the environmental impact of agriculture can be reduced by farming more intensively, with higher yields, on smaller areas of land (Waggonner et al., 1994). From this perspective, greenhouse gas emissions from Canadian agriculture could be minimized by increasing productivity on current lands, leaving more of the total land base in a 'natural' state. An example of this principle is the role of native grasslands as a storehouse of carbon. A century ago, much of this grassland was plowed, releasing large amounts of CO_2 – roughly equivalent to 10 years of fossil fuel CO_2 emissions at 1990 levels. Preserving the still-remaining grasslands, therefore, avoids further releases of CO_2 (Schuman et al., 2000). And if farming on existing cultivated lands can be intensified, it may even be possible to return some of these marginal arable lands back to grassland, thereby removing CO_2 from the atmosphere (Bruce et al., 1999).

However we choose to reduce emissions, we want to be wary of reduced productivity, especially if that shifts production (and associated greenhouse gas emissions) elsewhere. Measuring greenhouse gas emissions per unit of product may help avoid that pitfall.

3. Consider other benefits of practices that reduce emissions

A new mitigation method, though it may drastically reduce emissions, will have little effect unless it is widely adopted. And it will be widely adopted only if producers (and society) see benefits aside from reduced emissions. Despite exemplary altruism, farmers and ranchers may be hard-pressed to adopt new practices just to reduce emissions, especially if there is a cost attached. Consequently, a proposed new practice cannot be evaluated simply on its capacity to reduce emissions; it is not enough to ask: How much does a new practice reduce emissions? We also (and perhaps first) need to ask: How does it affect long-term ecosystem performance? What is the effect on other environmental indicators – biodiversity, water quality, air quality? How practical and feasible is its adoption? And, not least, how does it affect productivity and profitability? A practice that excels in reducing emissions but falters in these other questions is not likely to be adopted and its effectiveness in reducing emissions, therefore, is minimal.

Fortunately, many of the practices advocated for reducing emissions do have other benefits. Increasing soil carbon often promotes soil health and productivity; reducing CH_4 emissions from beef animals means higher weight gains per unit of feed ingested; reducing N_2O emissions from farm fields implies higher fertilizer use efficiency; reducing CO_2 emissions by conserving energy lowers costs of production.

Practices that reduce greenhouse gas emissions may have other tangible benefits. Often, it will be these other benefits that drive the adoption of mitigation practices – and the reductions in greenhouse gas emissions become a secondary, bonus reward. A keystone of research aimed at reducing emissions, therefore, is to consider the ancillary advantages of proposed practices (or, perhaps better, to view reduced emissions as an ancillary benefit of practices better for other reasons).

4. Follow the full carbon and nitrogen cycle

Farmers and ranchers, in effect, are managers of carbon and nitrogen. They rely on photosynthesis to trap carbon in organic forms, and then export that organic material, often enriched in nitrogen (protein), for profit. As a result, they depend on the continual flow of carbon and nitrogen through their ecosystems. And how they manage their farms and ranches, in turn, influence the carbon and nitrogen cycles within and beyond their land.

One approach in looking for ways to reduce greenhouse gas emissions, is to scrutinize the full C and N cycles, looking for obvious pressure points. For example, large beef feedlots rely on import of feeds (and nitrogen) from outlying farms, sometimes hundreds of km away. Because most of this nitrogen is excreted on-site, nitrogen from far-flung areas is effectively funneled into a small area. Unless there is some way of re-distributing it, the nitrogen will build up creating conditions for excessive loss of N_2O (and also the loss of fertilizer value). In this example, the way to reduce N_2O emissions may be to look at the larger N cycle, and find ways of avoiding the build-up of nitrogen near the feedlots.

5. Evaluate the potential mitigation practices over time

The extent to which a new practice reduces greenhouse gas emission may change with time. For example, adopting no-tillage on farmland may sequester carbon for one or several decades. But the benefit then diminishes as the soil approaches its maximum storage for that new farming method. In contrast, a practice that reduces CO_2 emission from fertilizer manufacture or reduces CH_4 emission from beef cattle may continue indefinitely, as long as that practice continues. It is even possible that a practice, which effectively reduces emissions 40 years from now requires an initial investment of energy that actually increases emissions in the short term (Fig.2). Consequently, the relative effectiveness of these various illustrative options depends on the time frame of interest. The method which is most effective now may not provide the best long-term reduction in greenhouse gas emissions.

Including time in our evaluation becomes even more important (and more complicated) when we realize that conditions may change in the foreseeable future; the climate may become warmer, precipitation may be altered, atmospheric CO_2 (and hence photosynthesis) will be changed. In thinking about ways of reducing emissions, we need to ask, not only 'How effective are they now?' but also 'What will be their cumulative effect over the next 10 years? Over the next 100 years?' It is an emerging irony, that in looking for ways to prevent climate change, we

need to prepare for climate change; we need to assess whether our preventative measures themselves will be influenced by the climate change they seek to forestall.

Implications

Climate change has now become firmly established in scientific thought. Despite lingering uncertainty about its effects – indeed partly because of them – Canada and other nations have committed to reducing emissions of greenhouse gases. There is now enough momentum, it seems, that reducing emissions will be a prominent objective for years to come. We have few options now but to accept that challenge, each in our own sectors of society.

Agriculture can help reduce emissions in several ways: by limiting its own emissions, and by creating carbon sinks and biofuels. Given growing pressure to reduce emissions, we may be tempted to scurry about, focusing on ways to suppress the flux of gases, trying various practices, alone and in combination, to see which best stems the flow. This approach may have merit. But a broader alternative is to use greenhouse emissions as markers of inefficiency. Where we see an excessive release of greenhouse gases – CO_2, CH_4, N_2O – it often points to an inefficiency in production. High N_2O losses imply inefficient use of expensive nitrogen, excessive CH_4 emission means poor use of feeds, excessive CO_2 points to inefficient energy use or waste of stored carbon. By eliminating the efficiencies, our ecosystems run more smoothly, perhaps more cheaply – and emissions are reduced. Rather than scrambling for ways of reducing greenhouse gas emissions, we can use these emissions as indicators of inefficiencies – and correcting these inefficiencies may improve productivity, enhance ecosystem health, reduce costs and, as a very desirable secondary effect, also reduce greenhouse gas emissions.

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Table 1: Atmospheric concentrations of CO_2, CH_4, and N_2O, the greenhouse gases most influenced by agricultural activity (from IPCC 2001).

Gas	Pre-industrial concentration	Concentration in 1998	Rate of increase (1990-1999)	GWP§
CO_2	~ 280 ppm	365 ppm	1.5 ppm/yr	1
CH_4	~ 700 ppb	1745 ppb	7.0 ppb/yr	23
N_2O	~ 270 ppb	314 ppb	0.8 ppb/yr	296

§Direct global warming potentials, relative to CO_2, for a 100 year time horizon. For example 1 kg of CH_4 emitted has a global warming contribution equal to that of 23 kg CO_2 over a 100 year period. This value is used to convert greenhouse gas emissions to CO_2 equivalents.

Table 2: Estimates of greenhouse gas emissions from Canadian agriculture in 1990 and 1998 (UNFCCC 2001). These estimates do not include all CO_2 from energy use on farms; most of these emissions are included in other categories in international greenhouse gas inventories.

Source	Gas	1990	1998
		Million tonnes CO_2 equivalent	
Ruminant livestock	CH_4	16.0	18.0
Manure management	CH_4	4.6	5.1
	N_2O	4.2	5.0
Agricultural soils	CO_2	7.3	0.9
	N_2O	36.0	40.5
Total (Agriculture)		68.0	69.5
Total (All sectors)		602.9	683.8

Table 3: Examples of practices proposed to reduce greenhouse gas emissions from various facets of agricultural systems. (Compiled from various sources, including: Mosier et al., 1998a,b; Cole, 1996; Janzen et al., 1998).

System component	Greenhouse gas	Proposed practice
Soil management	N_2O	• Use better fertilizer forms and methods • Match rates and time of N application to plant needs • Use slow-release fertilizers and nitrification inhibitors • Manage soil water (avoiding water-logging)
	CO_2	• Reduce tillage • Intensify cropping systems (e.g., less fallow) • Plant more grasses and other forages
Animal management	CH_4	• Improve feed quality and digestibility (e.g. high grain diet) • Add feed supplements (e.g., ionophores, oil) • Modify rumen microflora, genetically or with vaccines • Improve animals through genetics
Manure management	CH_4	• Improve aeration in manure handling systems • Minimize bedding in manure • Cool liquid storage tanks • Apply manure to land as soon as possible • Cover lagoons
	N_2O	• Apply manure at rates commensurate with crop N needs
Energy Use	CO_2	• Reduce tillage • Use legumes in place of N fertilizers • Grow crops for ethanol production • Collect CH_4 from manure (replacing fossil fuel use)

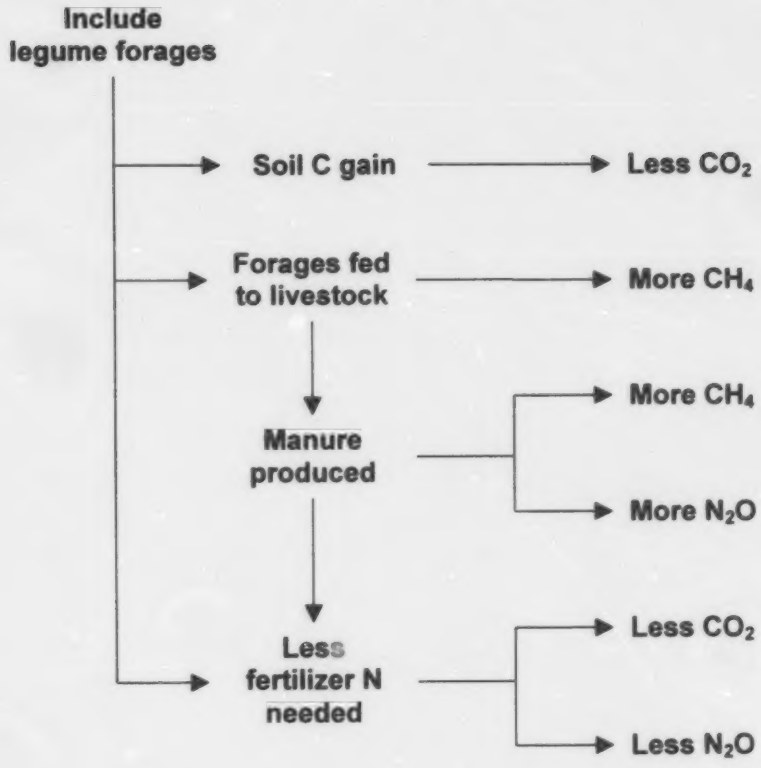

Figure 1: Conceptual flow diagram showing how adoption of a new practice, introducing forages into a crop rotation, affects greenhouse gas emissions throughout the entire farming system. To calculate the net effect of that practice on greenhouse gas emissions, we need to consider effects on all gases for the whole system.

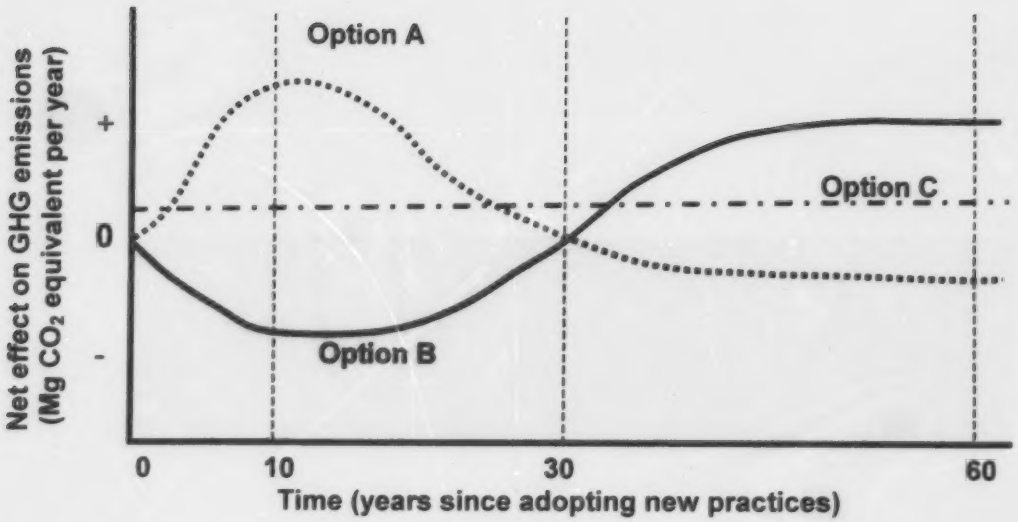

Figure 2. Diagram showing how the effect of mitigation practices may vary over time. With Option A (e.g., adoption of some C-sequestering practices), there are high short term benefits, but eventually increases in C storage cease, and the energy needed to maintain the stored C means that the net effect on emissions falls below zero (though the cumulative effect is still positive). With Option B (e.g., production of bio-fuels) there may be high greenhouse gas emissions in the short-term, as infra-structure is being developed, but eventual benefits over the long term. With Option C (e.g., use of energy conservation techniques or rations that reduce CH_4 emission), there is a consistent, cumulative effect over the long term. Though over-simplified, this diagram illustrates that the 'best' option for reducing emissions depends entirely on the time period considered.

A Feedlot Nutrient Map

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Key Points

- A feedlot concentrates nutrients; less than 20% of ingested nitrogen (N) and approximately 25% of ingested phosphorus (P) is retained by the animal. The majority of the excreted N is volatilized (over 60%) and most of the excreted P remains in the manure stockpile (over 70%). Therefore, management of the nutrients is required from both the environmental as well as the production perspective.

- The form and stability of the excreted nutrient significantly influences the point in the system for potential environmental damage.

- N is volatile because approximately 50% of excreted N is in the soluble form. Therefore, N is susceptible to loss from all parts of the system.

- P is stable and mostly excreted in an insoluble form in the feces and therefore the greatest potential environmental damage is a build up of P in the soil in the long-term.

Introduction

Rations are formulated to meet the protein and energy requirements of the animal. As well as nitrogen (N) the ration contains other nutrients, such as phosphorus (P). Not all of the protein and nutrients fed to the animal remains in the animal; any excess of requirements is excreted in manure, urine or as a gas. The form and stability of the excreted nutrient influences the point in the system for potential environmental damage.

Several models have been built that predict the nutrient balance of one or more aspects of an animal production system (e.g., BEEFBAL (1992), DAFOSYSM (2000), FSIM (Lott, 1998), MCLONE (1999) and MEDLI (1997)), but rarely do they consider the ration inputs and most are not a dedicated feedlot model. A software tool, FeedNuts, has been developed at the Lethbridge Research Centre to predict the nutrient inputs to the animal, quantify the amounts excreted and simulate their subsequent fate. FeedNuts considers the variation in the system by requiring the inputs into the model to be in the form of a distribution, and therefore, the model predictions are also distributions of likely outcomes, rather than a single value.

Model Description

The predictions of the nutrient balance in the FeedNuts model require the characteristics of the ration to be known. These characteristics are obtained from several feed databases including the database of feed ingredients for Southern Alberta (Corbett, 1997). Once the total ration has been mixed the average and standard deviation of the characteristics of that ration are calculated, including

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Total N and P concentrations, dry matter percentage, dry matter digestibility, total digestible nutrients (TDN) and undegradable intake protein (UIP) (or bypass protein).

The total nutrient excreted by the animal daily is the difference between that ingested and that retained by the animal. The predicted retention of N and P is based on retained energy calculations (NRC, 1996). The partitioning of the excreted nutrient into soluble and insoluble pools requires a simple input of this percentage for P, while the predicted partitioning of N is based on a metabolizable protein system, adapted from NRC (1996). The changes in the mass balance once the nutrient has been excreted are calculated daily after predicting volatilization and mineralization of N and losses of all the nutrients in runoff. Volatilization is the loss of ammonia to the atmosphere and mineralization is the process of converting organic N to an inorganic form.

The FeedNuts model incorporates a database of the inputs and can be used to store a record of the animal groups that have passed through the feedlot. Simple mass balance calculations on this database provide an estimate of the quantity of nutrients within each of the subsystems of the feedlot at any point of time.

Using the Model to Investigate the System

The model was used to investigate the variation in the feedlot system, the effects of weather and changing the characteristics of the ration. The effects of these changing conditions on the overall mass balance of the feedlot were investigated after animals were fed for approximately one year. The animals entered the feedlot on January 1st and the simulation was completed on the December 31st.

A barley based diet, consisting of 8% silage, 88% grain and 4% supplement on a dry matter basis (number 6 in Table 1) was used to investigate the mass balance of N and P on the 31st December. The animals entered the feedlot at 219 kg and finished at 517 kg, for a total feeding time of 337 days, and therefore for the last 28 days of the simulation no animals were in the feedlot.

The model predicts that when animals are fed the 8% silage barley based diet, the amount of N retained by the animal is a small percentage of the nutrient ingested (17%) with the majority of the N being volatilized (64% of ingested) and 4% being lost in runoff, leaving only 15% if the ingested N in the manure stockpile at the end of the year (see Figure 1a). The 15% remaining in the stockpile equates to 2.4% total N content of the manure (dry basis) with approximately ¼ in a soluble form (assumed to parallel the inorganic N) and the remaining ¾ in an insoluble form (assumed to parallel the organic N). The remaining soluble N is susceptible to further volatilization and as the insoluble form is mineralized over time it also is available for volatilization.

The amount of P retained by the animal is 25% of the ingested P. The majority of the P remains in the manure stockpile (74%), most of which is in a stable insoluble form and the total amount of P predicted to be transported in runoff is only 1% (Figure 1b). The P remaining in the stockpile equates to 1.98% total P in the manure (dry basis), which is high when compared to literature values. Eghball and Power (1998) quote 0.65% as an average total P in dry feedlot manure with values ranging from 0.12% to 1.6%. The high predicted value is partly a function of the high total P content of Southern Alberta feedstuffs used for the 8% barley silage ration (Table 1).

The high predicted total P compared with literature values might also be a result of soil being incorporated in the manure when the pens are cleaned. The mixing of soil with the manure pad is not included in the model, but would have the effect of decreasing the nutrient percentages in the sampled manure.

Variation in the System

The variation in the system was investigated by running a Monte Carlo simulation (20 simulations) for one year using 20 weather sequences generated with statistics from 40 years of weather data for Lethbridge (Richardson, 1981). A Monte Carlo simulation utilises sequences of random numbers to determine the input values based on the normal distribution characteristics for each input. The model is run 20 times for each scenario producing output in the form of a distribution. The daily variation in the amount ingested, excreted, moved in runoff and volatilized from the pen is investigated by summarising the predicted values over the 1-year time period for the 8% barley silage based diet.

The coefficient of variations in the 8% silage diet, which is a typical feedlot finisher ration in the Southern Alberta region, for total N and total P are 16% and 20%, respectively. This variation coupled with the variation in dry matter intake, digestibility of the diet and the other variables that are inputs to the model results in the variation in the various N pathways shown in Figure 2a. The coefficient of variation for the ingested N is about 44%, while the coefficient of variation for total excreted N is about 50% (a 6% increase in the variation). The coefficient of variation of the N volatilized daily from the pen is 97%. However, as is evident from Figure 2a there is considerably more variation in the amount of N moved in the runoff on any given day that runoff events occur.

The skewed distribution for N volatilized from the pen (Figure 2b) is a result of volatilization being modelled as a function of temperature and moisture content of the manure pad. When the temperature is below 5°C no volatilization occurs, but volatilization increases as the temperature and moisture content increases (Eghball and Power, 1998). Therefore, when the moisture and temperature conditions are favourable a considerable amount of N can be lost via volatilization on any given day. The skewed distribution for the N moved in a runoff event, indicates that most events are relatively small but there is a potential for a large amount of N to be moved in one event (the maximum predicted is approximately 1.7 kg/hd/d).

The variation throughout the system of the total daily P partitioning is shown in Figure 2b and indicates that in absolute terms the variation is not as great as for N, however in percentage terms the coefficient of variation of ingested P is 40% and the coefficient of variation of excreted P is 46% (also a 6% increase). Again the variation in the amount of P lost in runoff is greater than the daily ingested and excreted variation, with a coefficient of variation over 500%.

Weather Effects

The effect of weather on the overall mass balance was investigated by carrying out 20 Monte Carlo simulations for three prevailing climate scenarios. Twenty weather datasets for input into the Monte Carlo simulations were generated for each of the three climate scenarios based on the statistics for each scenario; a 20 percentile dry year, a 80 percentile wet year and an average year.

The same 8% silage ration and animal performance data (number 6 in Table 1) used to investigate the variation in the system were used to investigate the effects of weather. Figure 3 is a comparison of the N balance in each of the compartments over the time period simulated for each of the climate scenarios. The biggest difference is in the amount of N volatilized from the manure pad and stockpile (230 kg, 251 kg and 262 kg for 10 head for wet, average and dry years respectively). However the total soluble N in the runoff over the year is 37 kg, 17 kg and 3 kg for 10 head for the wet, average and dry years respectively. Therefore the biggest effect of the prevailing climate is to change the partitioning of the soluble N between the volatilized and runoff pools. These differences

do not affect the overall N content of the manure stockpile at the end of the year, which is around 2.3%-2.4% (dry basis) for all three scenarios (Figure 4a).

There is also little difference for the P content of the manure when the comparisons are made on a dry basis, which is approximately 2% for all scenarios (Figure 4a). However, when the nutrient content of the manure is compared on a wet basis the concentration increases by approximately 1% for both N and P from a wet year to a dry year (Figure 4b).

Eight tonnes/ha of manure with an N content of around 2.3% is required to be applied to a Southern Alberta land base on a dry basis to meet the N requirements of one barley crop in an average year. The calculation of this application rate does not consider any residual nitrogen in the soil or any losses from leachate or runoff. If this same rate is used to apply the manure from a wet year there would be a deficit of 3.3 kg N and if applied at the same rate from a dry year there would be a surplus of 4.2 kg of N (Figure 4c). Therefore, when manure is applied on a dry matter basis there is little difference in the N soil balance when comparing manure from wet, average and dry years.

Barley grown in Southern Alberta removes approximately 6 times more N than P and therefore for the three weather scenarios there is considerably more build up of P in the soil (123 kg, 125 kg and 130 kg for manure from wet, average and dry years respectively). However, when the manure is applied on a wet basis a different balance is evident (Figure 4d), with the rate of application of manure from an average year required to meet the N requirement of one barley crop being 28 tonnes/ha (wet basis), which would result in a deficit of 71 kg N if the manure from a wet year was applied at the same rate, but the manure from a dry year would result in a surplus of 234 kg N. The surplus of P increases by 258 kg when the manure is applied from a dry year compared with the manure from a wet year (Figure 4d).

Figure 4 also highlights the differences in the variability of the predicted N and P contents, which is a result of the volatility of N and the greater pool of soluble N compared with soluble P. The decreased variability of the N content in a wet year on a wet basis (Figure 4d), is a function of the predicted moisture content of the manure pad being fairly constant for the wet year simulations. Figure 4 also indicates the variation in the manure concentrations is reduced when considered on a dry basis and as the moisture content of the manure pack is a relatively easy thing to measure a more precise estimate of the nutrient balance in the soil can be estimated when calculations are done on a dry basis.

Ration Effects

The rations used in a study of the influence of the ratio of barley silage to barley grain on rate and efficiency of liveweight gain, diet digestibility and carcass characteristics of beef steers (Hironaka et al., 1994) were used to investigate the effect of diet on the mass balance of N and P. The characteristics of each of the ingredients in the rations were obtained from the Southern Alberta feeds database (Corbett, 1997) and the predicted characteristics of the total mixed ration and other parameters required as inputs to the model are shown in Table 1. Two periods were defined in the experiment of Hironaka et al. (1994). In the first period the quantity of feed offered was restricted to an amount calculated to obtain 0.6 kg/d of gain and for the remaining time the animals were fed to appetite.

Monte Carlo simulations (20 simulations) for a 1-year time period were run using average climatic conditions for the Lethbridge area and for each of the rations in Table 1 (Hironaka et al., 1994). The effect of the ration on the overall nutrient mass balance of the feedlot system was investigated, particularly the effect of the energy content of the diet. Because the diets fed in the

Hironaka et al. (1994) experiment ranged from 0% to 100% silage the finishing times for each of the groups was different and for the diets with higher percentages of silage the animals did not always finish in the 1-year time period. Therefore, the effects of different levels of silage in the ration are investigated over a 1-year time period and also by considering an animal weight endpoint, which included all days up until the time the animals reached 400 kg (Figure 5a).

The trend in the nutrient mass balance was different only for the 100% silage diet when comparing the animal weight endpoint (Figure 5a) versus the 1-year time period (Figure 5b). As the percentage of silage in the diet decreases the total N ingested decreases because the N content of barley silage is higher than that of barley grain. Due to reduced N intake, N excretion is also reduced. The silage content of the diet also affects the form of the excreted N; with higher grain diets, the insoluble N excreted in feces decreases and the soluble form in urine slightly increases. Reduced total N intake due to the lower N content of the barley grain compared with silage is the main reason for reduced insoluble N. The increased excretion of soluble N is due to the increased intake of UIP, because barley grain has a higher UIP content compared to silage. The UIP is absorbed from the gut by the animal, metabolized and is potentially excreted in urine. When N intake exceeds the requirements of the animal, as in the case of these diets, increasing intake of UIP increases excretion of metabolized N in the soluble form.

The effect of the level of silage in the diet has no effect on the P removed in the runoff (not shown), however there are some differences in runoff N (Figure 5c) with small increases with a decreasing percentage of silage in the diet up to the 8% silage. The non-linear trend for N in runoff is associated with the volatility of N and also the form in which it excreted (i.e. soluble versus insoluble).

The increase in the average amount of N in the runoff parallels the increase in the N in the soluble form excreted by the animal. However, this same trend is not evident for the volatilization of N (Figure 5d), which is an unexpected result. The volatilization of N is the major process operating on the changing pools of N on any given day and is modelled as a function of temperature and moisture content of the manure pad. The N available for volatilization comes from the soluble pool, which is made up of the excreted soluble N plus the N that is mineralized from the insoluble pool daily. The mineralization of the insoluble component is a function of temperature and is based on the algorithms from a previously published model (GISMaize, 2000). There is more mineralization in the high silage diets because of an increasing insoluble N pool with increasing silage contents. This greater mineralization changes the relative amounts of N available for volatilization over the course of the year as a function of percentage of silage in the ration.

The volatility of N and the net transfer from the insoluble to the soluble pool through the mineralization process further complicates the prediction of the manure characteristics at the end of the year. As a large proportion of the soluble N is 'lost' by volatilization or in runoff and the total insoluble excreted component increases with an increasing percentage of silage it is reasonable to assume that the total N content of the manure also follows the same pattern (i.e., an increasing N percentage with an increasing percentage of silage). However, as can be seen from Figure 6a, this is clearly not the case, with very little difference seen in the N content of manure as a function of proportion of silage in the diet. Apart from mineralization, another factor affecting this outcome is the total amount of solids excreted, which is a function of the digestibility of the ration. At the end of the year the 100% silage diet is predicted to produce 3 tonnes more dry matter than the 0% silage diet, which tends to balance out the increasing insoluble N excretions from the increasing silage percentages when the manure characteristics are considered on a percentage basis.

Because of the stable nature of P the prediction of the manure characteristics are not as complicated as for N, however the amount of solids excreted does influence the predicted outcomes on a percentage basis (Figure 6a). Each ration had the same P content (see Table 1), however there were varying dry matter intakes and average daily gains from period 1 to period 2. Generally, over the course of the year there was more P ingested for the higher silage rations, except for period 2 for the 100% silage ration because of a lower dry matter intake. There was more P retained for the lower silage diets and therefore less excreted, and therefore the P content of the manure could be expected to decrease as the percentage of silage in the diet decreases. However, the total solids excreted have a greater influence and the P content of the manure actually increases with a decreasing percentage of silage in the ration, even though the total amount of P decreases (Figure 6a).

The manure from the different percentage of silage diets was applied at the rate to supply the total N requirements of one barley crop for Southern Alberta using the manure from the 8% silage diet. This application rate (8 tonnes/ha on a dry matter basis) resulted in a deficit of N for all of the other treatments and a build up of P for all treatments ranging from approximately 50 to 125 kg/ha (Figure 6b). However, it is unlikely that the total N and P will be available throughout the growing season. Assuming that only the soluble component of P is available there would not be sufficient P for the crop if the manure was applied at the same rate (Figure 6c).

Conclusions

The use of the FeedNuts model to investigate the system highlights the areas where control of the nutrient balance can be obtained. The prevailing climate in a particular year does have some effect on the overall mass balance of the system but of course cannot be controlled. However, the effect of the percentage of silage in the diet appears be more significant, especially on the P content of the manure. As the percentage of silage in the diet decreases, the percentage of P in the manure increases. The percentage of silage in the diet is an input that can be controlled to a large degree.

The variability in the nutrient content of manure from feedlots is a function of the variability in the rations, feed intakes, animal growth, climate and the hydrological cycle. Therefore predicting the nutrient content of the manure as a function of different rations is not a trivial task. FeedNuts predicts the nutrient content of the manure and the mass balance in different parts of the system and therefore provides an inexpensive tool to evaluate the effects of changing management and feeding strategies.

This exercise has highlighted the areas where more data are required to validate the predictions, particularly manure data as a function of different feed inputs and also the predicted high P content of manure for low silage diets. The relatively low percentage of N and P retained by the animal highlights that careful management of the excreted nutrients is required to obtain the maximum benefit throughout the system from the initial feed input.

Implications

In the United States, the confined animal feeding operations (CAFO) regulations are scheduled for publication by January 2006, and once final, these regulations will come into effect immediately (EPA, 2001). In essence operations falling under these CAFO regulations will be required to prepare and implement a site-specific permit nutrient plan. Regardless of whether Alberta follows these guidelines, it is clear that the nutrient balance of the feedlot is becoming an important component of the overall management of the feedlot. A pro-active approach by feedlot producers now in trying to address some of the areas where improvements can be made should provide a solid

foundation if regulations requiring the submission of nutrient management plans are imposed in the future. FeedNuts provides a tool to facilitate the pro-active approach required.

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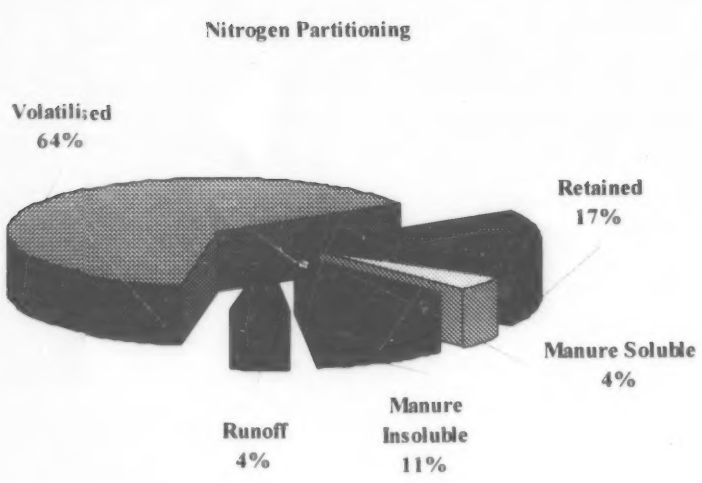

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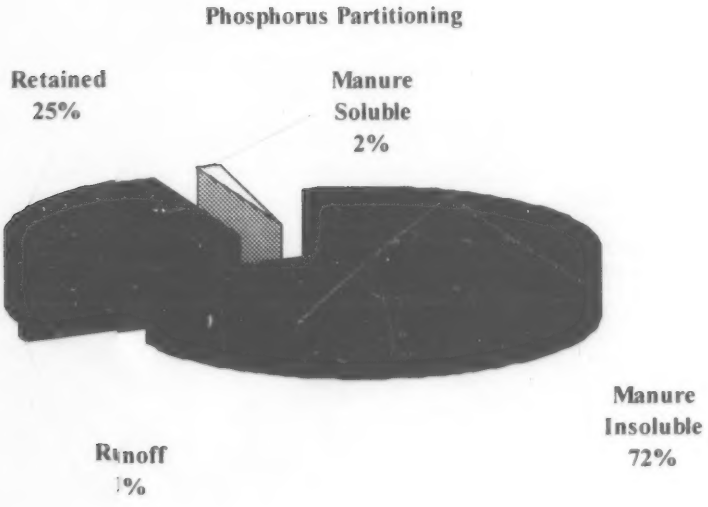

Figure 1. Predicted partitioning of a) nitrogen and b) phosphorus as a percentage of ingested nutrients after animals were fed an 8% silage diet for 337 days.

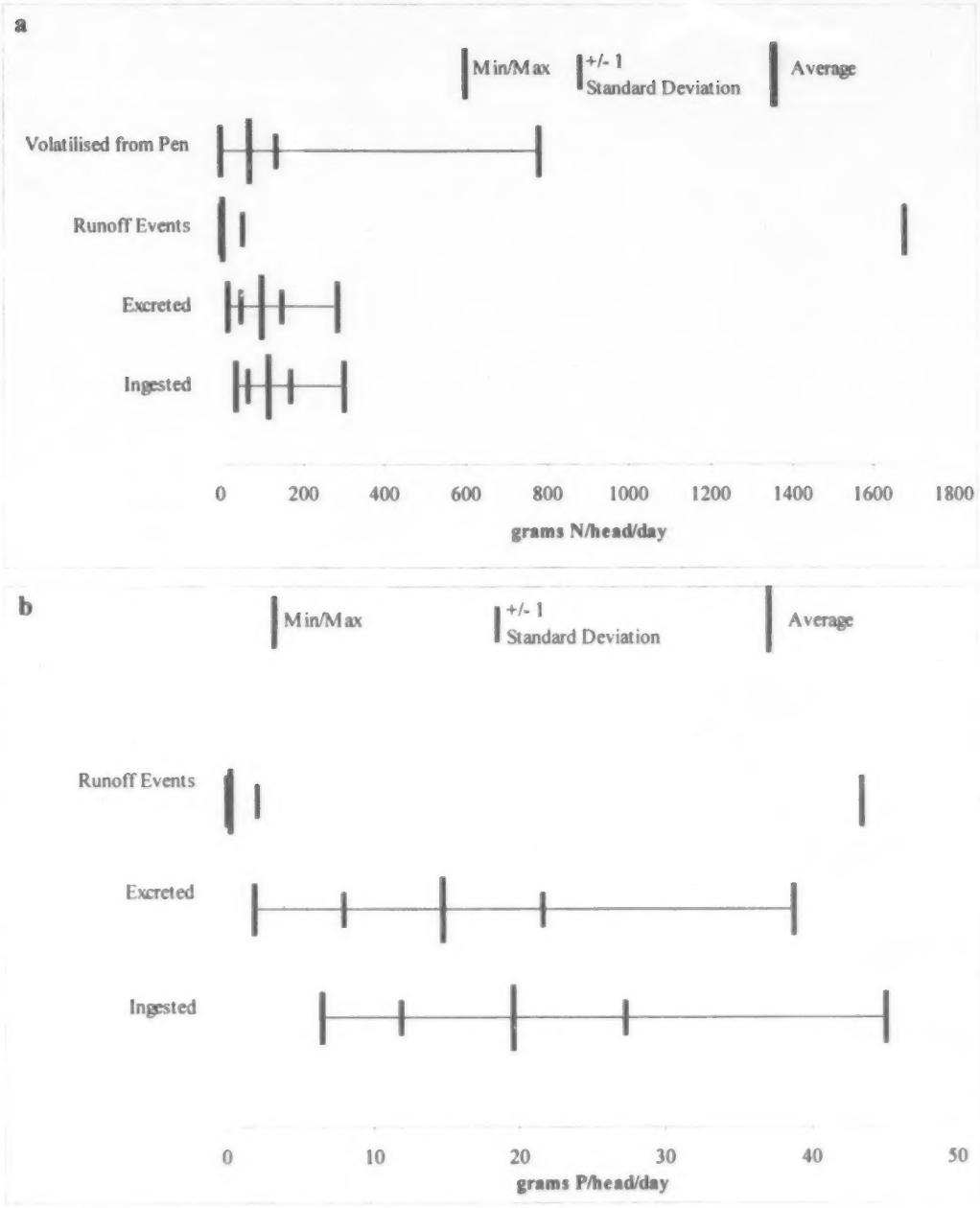

Figure 2. Daily variation in a) nitrogen and b) phosphorus pools in the feedlot system predicted from 20 Monte Carlo simulations (data are for a single animal).

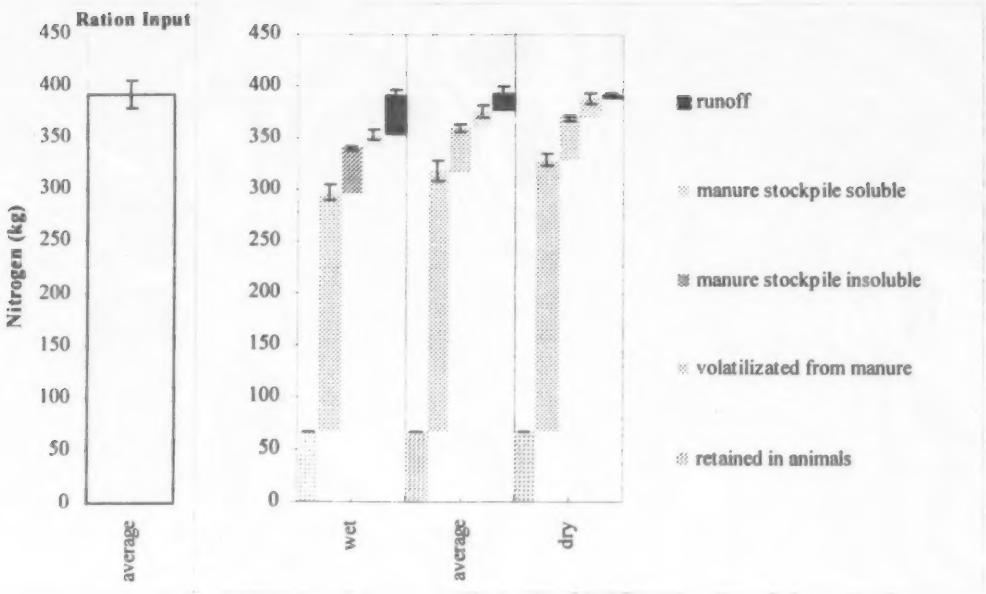

Figure 3. The effect of wet, average and dry weather on the overall nitrogen balance in the feedlot (data are for 10 head).

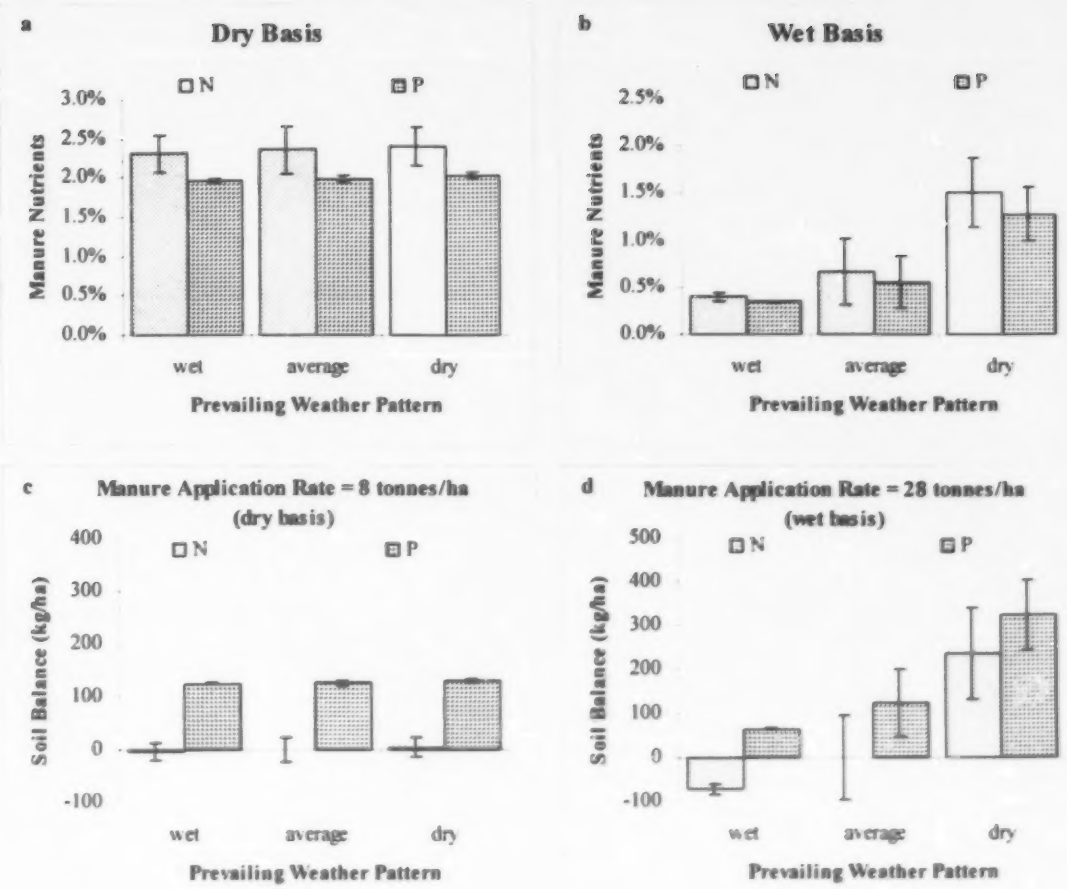

Figure 4. The effect of wet, average and dry weather on the nutrient content in the manure stockpile for the 8% silage diet expressed on a a) dry and b) wet basis and the nutrient balance in the soil from the subsequent application of that manure on a c) dry and d) wet basis (error bars are +/- 1 standard deviation).

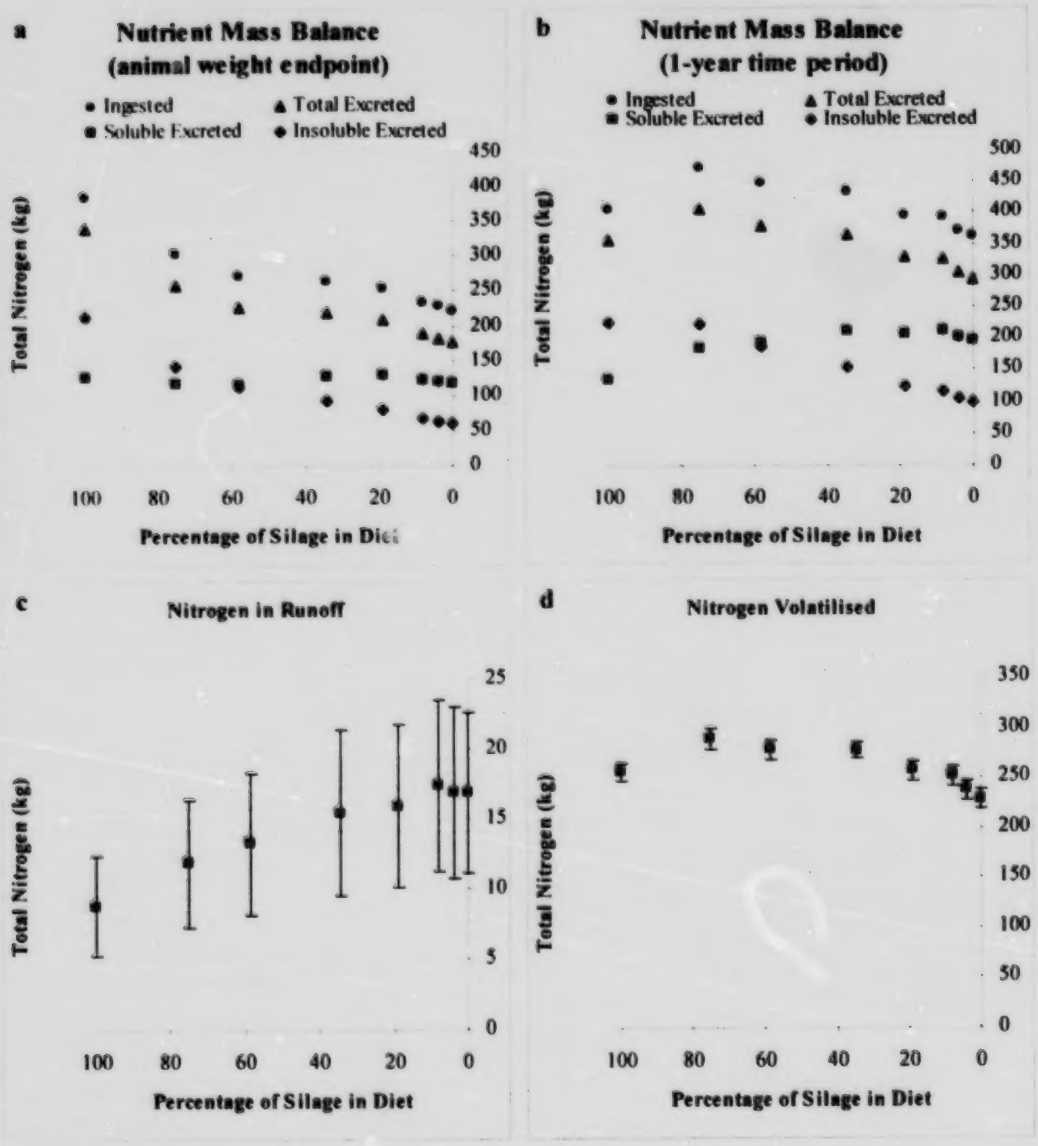

Figure 5. The animal mass balance for 10 animals for the period a) to attain 400 kg animal weight endpoint and b) a time period of one year, c) nitrogen removed in runoff and d) volatilized as a function of silage in the diet (dry matter basis).

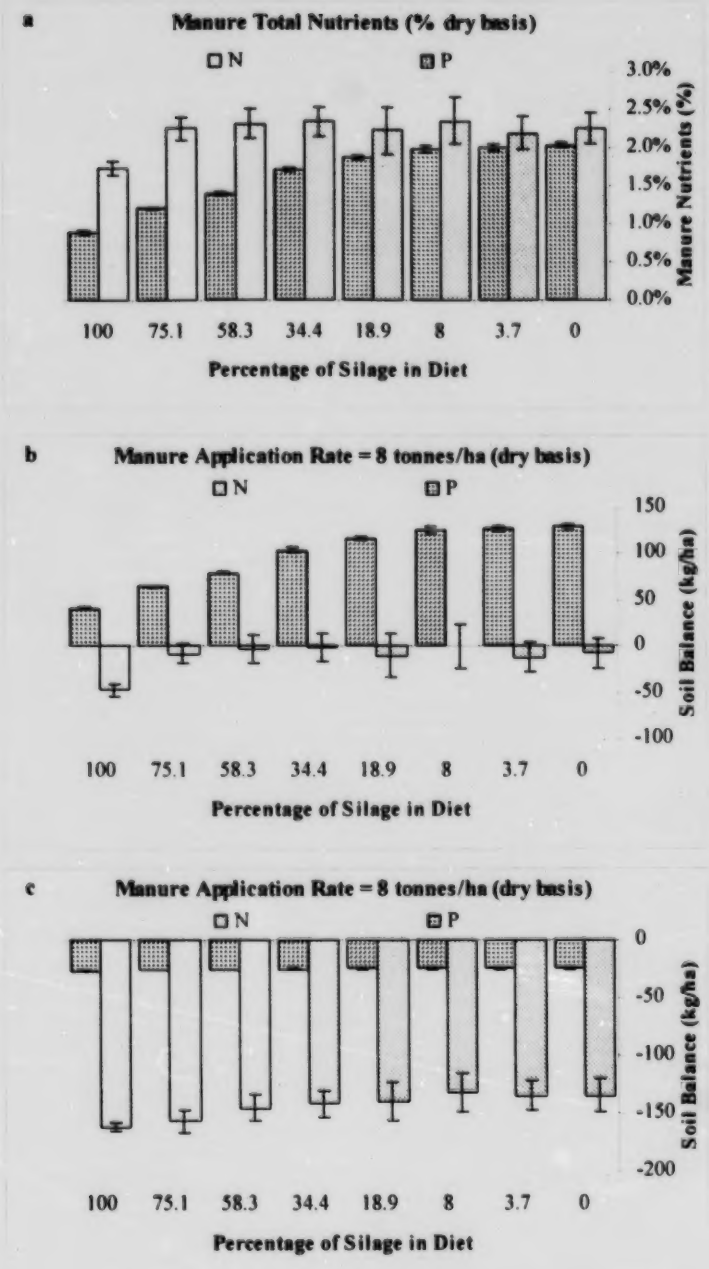

Figure 6. The effect of different levels of silage in the diet (dry matter basis) on a) predicted nutrient content of manure (dry basis), b) the applied total nutrients minus nutrients taken up by one barley crop and, c) the applied soluble nutrients minus nutrients taken up by one barley crop.

Table 1. Ration composition, animal performance and predicted characteristics of mixed rations ranging in level of barley silage (Hironaka et al., 1994)

	Ration Composition							
Ingredients	Diet							
(% DM basis)	1	2	3	4	5	6	7	8
Barley silage	100.0	75.1	58.3	34.4	18.9	8.0	3.7	
Barley sixrow		24.0	40.2	63.3	78.3	88.8	92.9	6.5
Canola Meal		0.1	0.2	0.3	0.3	0.4	0.4	0.4
Limestone		0.3	0.5	0.8	1.0	1.1	1.2	1.2
Molasses Beet		0.3	0.4	0.7	0.8	0.9	1.0	1.0
Salt		0.2	0.4	0.6	0.7	0.8	0.9	0.9
Parameter	**Predicted Ration Characteristics**							
Dry Matter, %	35.6	48.7	57.6	70.2	78.4	84.1	86.4	88.4
Total N, % DM	1.8	1.9	1.9	1.9	2.0	2.0	2.0	2.0
Total P, % DM	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
UIP1, % CP	14.0	19.0	22.4	27.2	30.3	32.5	33.4	34.1
TDN2, % DM	60.2	65.3	68.8	73.7	76.9	79.2	80.0	80.8
DMD3	75.8	78.9	81.0	84.0	85.9	87.3	87.8	88.3
	Measured Animal Performance							
Measure	**PERIOD 1**							
DMI4, kg/d	4.9	4.6	4.4	3.9	3.6	3.6	3.4	3.2
DWG5, kg/d	0.4	0.5	0.6	0.5	0.5	0.5	0.5	0.5
Entry Weight, kg	220	220	219	218	218	219	218	217
Exit Weight, kg	286	307	313	304	304	310	304	303
Number of days	168	168	168	168	168	168	168	168
	PERIOD 2							
DMI, kg/d	7.6	8.9	9.1	9.5	8.9	8.2	8.3	7.7
DWG, kg/d	0.6	1.0	1.2	1.3	1.3	1.2	1.4	1.3
Entry Weight, kg	286	307	313	304	304	310	304	303
Exit Weight, kg	410	514	519	523	506	517	524	523
Number of days	196	210	180	167	158	169	157	164

1UIP = Undegradable Intake Protein
2TDN = Total Digestible Nutrients
3DMD = Dry Matter Digestibility
4DMI = Dry Matter Intake
5DWG = Daily Weight Gain.

Session IV - Nutritional Considerations

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Vegetable Oil in Beef Cattle Diets

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Key Points

- Dietary oil affects animal performance through positive effects on microbial populations in the rumen.
- Dietary oil produces beneficial effects on secretion and effectiveness of intestinal hormones and decreases viscosity of intestinal contents.
- Dietary oil improves rate of digestion and absorption of nutrients in the intestine leading to positive effects on growth rate and efficiency of production.

Introduction

Fat is an energy dense nutrient and is an attractive feed component for use in feeding animals with a high demand for energy, such as dairy cattle and finishing beef cattle (Sutter et al., 2000). However, there is a limit to the extent that fat or oil can be added to ruminant diets. Many studies have indicated that fat (especially oils, which are liquids at room temperature due to the occurrence of double bonds in the fatty acids that make up oils) negatively affected fermentation in the rumen and digestibility of feeds (Flachowsky et al., 1995). This negative effect is most critical when high forage diets are fed to cattle. In order to circumvent the effect, fat was included in cattle diets after protecting it from degradation in the rumen (Sutter et al., 2000) or as a combination of prilled fat and canola oil (Jenkins and Jenny, 1992).

By-products of vegetable oil and canola oil refining called acidulated fatty acids are available to beef producers. They are widely used at approximately 4% of diet dry matter (DM) in ground forage diets to control dust (Beacom and Mir, 1985). These acidulated fatty acids are the free fatty acids that are stripped from canola oil during refining (Thacker and Campbell, 1987) and contain approximately 57% free fatty acids and 23% triglycerides, thus, they can provide substantial amounts of energy. Therefore, it was of interest to determine the direct effects of the acidulated fatty acids in ruminant diets on growth rate and to determine the maximum level of inclusion without jeopardizing animal performance. Studies were conducted by Mir (1988) to determine the effect of supplementation of ground alfalfa hay diets with increasing levels of acidulated fatty acids on sheep growth rate, production efficiency and rumen fermentation characteristics. Results (Table 1) indicated that addition of acidulated fatty acids did have a beneficial effect on performance of the sheep when included at up to 5% of diet DM. Inclusion rates of fat in excess of 5% have been implicated in negative effects on fibre degradation (Moore et al., 1987) through its inhibitory effect on microbial degradation of fibre (Mathison et al., 1998).

Although the use of beef tallow has been common, vegetable oil use in beef cattle diets has been relatively small. Recently the impact of dietary oil on enhancing the content of essential fatty acids and bio-converted fatty acids of importance to human health in meat and milk has rekindled interest in the use of vegetable oils in ruminant diets. Although forage lipid contains significant levels of unsaturated fatty acids (Van Soest, 1994), the fat content is not substantial and even at high daily intakes the contribution of fat from forages is relatively small. In order to increase the concentration of the essential fatty acids and especially the bio-converted fatty acids in ruminant products, it is necessary to add oil to ruminant diets. To enhance the concentration of the bio-converted fatty acids it is important that the oils are not protected from ruminal degradation, in order to enable the ruminal microbial enzyme systems to perform the bioconversion (Kepler et al., 1966).

Associated effects of dietary oil in ruminants

Reproduction

It is clear that oil in ruminant diets has many effects on overall performance of the animal. A high proportion of the fatty acids from flax seed are the omega -3 (ω-3) fatty acids or the linolenic acids. Linolenic acid has three double bonds in its structure and the first double bond is between the 3rd and 4th carbon of the fatty acid, when the carbon atoms are counted from the -CH_3 end of the fatty acid. The prostaglandins that are formed from linolenic acid appear to have different functions and effectiveness than those formed from linoleic acid, an ω-6 fatty acid (Fly and Johnston, 1990). Petit et al. (2001) found that inclusion of 17% flax seed in dairy cow diets improved conception after first insemination (artificial) from 50 to 87% (Table 2). Also the converted fatty acid of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) which are derived from ω-3 linolenic acid have biological functions in neurotransmitter effectiveness at neuro-muscular synapses and improvement of visual acuity. The EPA and DHA can be synthesized by mammalian enzymes but there appears to be added benefit by augmenting their availability in humans through the food consumed.

Methane production and rumen protozoal populations

Dietary oil in ruminant diets has a positive effect on production through a number of factors. An estimated 15% reduction in methane emissions from beef cattle has been attributed to dietary oil, which was included at the level of 4 to 5% of dietary DM in high grain diets fed to finishing beef cattle (Wittenberg et al., 2001). The reduction in methane production is due to direct and indirect effects. Among the direct effects it is known that fats and oils, especially coconut oil, in cattle diets are toxic to methanogenic bacteria even in defaunated situations (Dohme et al., 1999) and the avenue for removal of H_2 is depressed due to low methanogens. The regeneration of NADH to NAD for glycolysis to proceed is depressed, thus production of other energy yielding compounds declines (Pavlostathis et al., 1990) leading to declines in production of substrates that support alternate methanogens. As a result, overall fermentability of the feed in the rumen declines (Mathison et al.,1998) leading to a reduction in methane production.

Among the indirect effects on methanogenesis, oils high in C18 fatty acids are toxic to protozoa (Newbold and Chamberlain, 1988; Machmuller and Kreuzer, 1999, Machmuller et al., 2000), which provide the H_2 for the methanogens' use. Rumen protozoa hinder energy and protein availability to the animal. The losses in energy are due to wasteful consumption of bacteria by protozoa, not allowing bacterial biomass to flow out of the rumen for the use of the host animal. Also, the protozoa harbour the

methanogenic bacteria (Hegarty, 1999), therefore the reduction of the protozoal population indirectly reduces methane production in vitro by 9 to 25% (Newbold et al., 1995).

More recently Ivan et al. (2001) have shown that unlike many other natural defaunating agents, sunflower oil at 6% of dietary DM fed to sheep reduced protozoa numbers significantly for up to 49 days from 7×10^5/ml to $< 2\times10^5$/ml (Figure 1). The pH in the rumen was elevated in oil fed sheep in comparison to sheep provided oil-unsupplemented diets. The diet in this study was a high barley silage diet and studies are under way to determine the effects in finishing diets, where the protozoa numbers are naturally lower than when animals are fed high forage diets (Weller and Pilgrim, 1974). The decreased protozoa numbers has advantages in maximizing protein nutrition of the host animal, because microbial nitrogen recycling in the rumen is decreased (Jouany, 1996) and the outflow of amino acids from the rumen to the small intestines is increased (Veira et al., 1984; Kayouli et al., 1986).

Intestinal digestion

Along with the unrestricted flow of amino acids to the host animal, the dietary oil also flows into the intestine. The fat content of duodenal digesta of corn fed cattle was greater than that of barley fed cattle because corn has a higher fat content (Mir et al., 2000a). In sheep fed oil-supplemented diets, viscosity of supernatant from intestinal contents collected at the proximal, mid and distal regions was lower relative to that of sheep fed control diets (Figure 2, Mir et al., 2000b). In rodents and swine, decreased viscosity of intestinal contents has facilitated both digestion in the small intestine and increased absorption of products of digestion (Blackburn and Johnson, 1981; Ellis et al., 1996). Reduction in small intestinal viscosity in poultry and swine through use of exogenous enzymes in diets resulted in improved feed conversion efficiency (Bedford et al., 1998; Yin and Baidoo, 1999). In beef cattle, provision of dietary exogenous enzymes decreased intestinal viscosity and the blood urea nitrogen was decreased relative to that in cattle not receiving the exogenous enzymes (Hristov et al., 1998). The elevation in small intestinal digesta viscosity due to increasing dietary grain (Mir and Mir, 1998) appeared to be a contributory factor to the absence of increase in portal plasma glucose in steers (Mir et al., 2000a; Kreikemeier et al., 1991).

In the study with sheep it was evident that dietary oil increased fat content of the small intestinal digesta and dry matter content of digesta was diminished in conjunction with the reduction in viscosity observed due to dietary oil (Table 3). A rapid decrease in amylase activity was observed in sheep fed oil relative to that observed in control sheep. This decrease in amylase activity may be related to destruction of amylase by the proteases secreted into the intestine (Table 4). In most species, the proteases are more abundant than amylase in pancreatic secretions (Lowe, 1994).

The proposed mechanism for viscosity reduction of small intestinal contents is through the ability of the oil or fat in the small intestine to induce the secretion of the hormone cholecystokinin (CCK) (Liddle, 1997). CCK is known to augment the hormonal action of secretin (Meyer et al., 1971) which activates bicarbonate and water secretion into the intestine, thus reducing viscosity of the contents. Partially hydrolyzed triglycerides are better triggers for secretion of CCK than intact triglycerides (Liddle, 1997). In ruminants, the dietary lipids are extensively hydrolyzed in the rumen unless the fat content of the diet is high, in which case the hydrolysis of the triglyceride is incomplete. Along with a substantial amount of free fatty acids other lipid classes are also found in the small intestinal digesta (Noble, 1981), offering improved induction of CCK secretion in ruminants compared to that in non-ruminants. Such improved stimulation of CCK secretion can be expected to lead to decreased viscosity of intestinal contents due to its effect on hormone release and effectiveness.

Apart from the effect that dietary oil has on triggering the hormonal release leading to improved digestion, there is evidence in other species that small intestinal mucosal mass is affected by dietary oils that provide essential fatty acids. In rats, the provision of an increasing proportion of essential fatty acids in the diet was found to increase intestinal biomass in mid and distal regions. The increase in biomass was corroborated with a concomitant increase in cell proliferation which was assessed by measuring 2 h metaphase accumulation in the intestine (Jenkins and Thompson, 1992). In mice, an essential fatty acid replete diet was better able to reduce bacterial translocation from lumen of the intestines to mesenteric lymph nodes than an essential fatty acid deficient diet. This indicated that oils which contain essential fatty acids afford better protection from bacterial infection (Barton et al., 1992).

The effects of dietary oil in the rumen and intestinal tract have a beneficial effect on nutrient utilization. Many reports show that addition of oil to diets has improved performance of cattle.

Dietary oil and animal performance

In a study that was conducted at Washington State University, Pullman, we found that supplementation of steer diets with sunflower oil at 6% of DM resulted in improved average daily gain (ADG) even though DM intake (DMI) was diminished relative to that observed for steers fed the control diet without the oil. This resulted in improved feed to gain ratio (F:G) in the backgrounding phase of the experiment (Table 5). During the finishing phase a relative improvement in ADG was observed but the decrease in DMI was not present, yet the results showed a small improvement in F:G (Mir and Mir, 1999).

In a trial conducted at the Lethbridge Research Centre with the support of Pioneer Hi-Bred International Ltd. the inclusion of sunflower seed at 9 and 14% of the dietary DM improved performance of finishing steers relative to those not receiving the sunflower seed (Table 6, Gibb et al., 2001). Although the value for ADG in control steers was low the relative improvement due to the oil seeds was significant. Furthermore, provision of the seeds as whole or crushed did not affect the performance of the steers. The use of dietary oil seed supplements is relatively less expensive than the use of oil supplements. Examination of feces indicated few whole seeds, indicating that supplementation with seed may be effective and less expensive than using extracted oil. Currently, most studies have employed oil in the diets and if crude feed grade oil is not available for use in animal feed, the cost can be prohibitive. However, either the use of oil seed or vegetable oil refining wastes may be less expensive than vegetable oils to use as dietary supplements for ruminants.

In a finishing trial conducted at Western Feedlots we found that steers fed a total mixed ration supplemented with a liquid product consisting of byproducts from vegetable oil refining and corn syrup manufacture at 12% of the diet had a tendency towards higher ADG relative to those not provided the supplement (Table 7). The actual feed intake could not be measured in these animals. Blood was collected after 84 days on the trial and blood urea nitrogen (BUN) was determined. Although the average BUN values in animals fed the supplement were only slightly lower than those observed for control steers, the range of the values for the animals fed the liquid supplement was smaller than that of the control steers. In non-ruminants a correlation has been described between F:G ratio and BUN (Owusu-Asiedu and Baidoo, 1999).

The low BUN has been suggested to indicate improved amino acid availability to the animal through decreased deamination and conversion of the resulting ammonia to urea for elimination. The pertinence of this parameter in ruminants needs validation because amino acid deamination in the liver

is not the only source of ammonia for urea synthesis in ruminants, but the rumen is a major source of ammonia. Also, the urea produced is recycled through saliva back to the rumen and is a major contributor to microbial protein synthesis, which is the source of amino acid nitrogen for the host animal.

The overview of the studies indicates a limit to the extent of oil that can be incorporated into ruminant diets without jeopardizing animal productivity. At optimum levels of oil inclusion in beef cattle diets an impetus to efficient growth rates can be achieved. This is due to the contribution of oil to dietary energy content, beneficial alteration of the ruminal microbial population resulting in fermentation products that are utilizable by the animal, and regulation of hormonal release and effectiveness resulting in improved nutrient digestion and absorption in the intestines, largely due to lower viscosity of intestinal contents.

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Table 1. Performance of lambs fed canola acidulated fatty acids in high forage diets.

Parameter	Canola acidulated fatty acids (%)				
	0	3	4	5	SEM
Average daily gain (g)	283b	307a	310a	329a	11.0
Dry matter intake (kg/d)	1.75	1.69	1.74	1.81	0.06
Feed:gain	6.2b	5.5a	5.6a	5.5a	0.2
Improvement over control (%)					
Gain		8.6	9.6	16.2	
Feed:gain		11.3	9.7	11.3	

Abstracted from (Mir 1988).

a,b Values denoted by different letters in a row are different ($P < 0.05$).

Table 2. Feed composition (as % DM) and production data of dairy cows fed Megalac (MEGA) or formaldehyde treated flax (FLAX) between weeks 10 and 20 of lactation.

Parameter	MEGA	FLAX
Diet component		
Silage	81.7	83.0
Megalac	5.6	0
Flaxseed meal	12.7	0
Flax seed	0	17.0
Diet composition		
Crude protein	16.7	17.0
Fat	9.7	10.0
Production parameters		
Dry matter intake (kg)	13.4	13.6
Body weight change (kg)	-61	-4
Milk production (kg/d)	19.8a	18.6b
Milk protein (%)	2.95	3.09
Milk fat (%)	b	a
Length of estrous cycle (d)	4.62	4.37
Conception at 1st AI, (%)	21.7	22.8
Milk progestrone µg/l	50.0a	87.b
	26.8	35.2

Abstracted from Petit et al., (2001).
Values denoted by different letters in a row are different ($P < 0.05$).
AI - Artificial Insemination.

Table 3. Composition of small intestinal contents from proximal, mid and distal regions of the small intestine in sheep fed diets with (+oil) or without (-oil) the dietary oil supplement.

Location in small intestine	Treatment	Dry matter (DM) (%)	Protein (% of DM)	Starch (% of DM)	Fat (% of DM)
Proximal	+oil	10.7±0.86ab	34.7±3.37a	0.73±0.92ab	11.52
	-oil	11.0±0.96a	36.6±3.37a	1.30±0.84ab	-
Mid	+oil	10.1±0.86bc	20.0±2.82b	1.08±0.65ab	8.9
	-oil	12.7±0.91a	20.4±2.97b	0.72±0.68b	7.3
Distal	+oil	8.2±0.86c	13.4±2.82bc	2.79±0.65a	3.5
	-oil	12.1±0.96a	10.9±3.15c	0.75±0.72b	5.2

Means in a column denoted by different letters are different ($P < 0.05$).

Table 4. Amylase activity in intestinal contents at proximal, mid and distal regions of the small intestine of sheep fed diets with (+oil) and without(-oil) the dietary oil supplement.

Location in small intestine	Treatment	Protein (mg mL^{-1})	Amylase activity	
			μM reducing sugars mg^{-1} protein min^{-1}	μM reducing sugars mg^{-1} DM min^{-1}
Proximal	+oil	28.3±3.29b	6.40±1.57a	2.03±0.40a
	-oil	38.4±3.29a	5.67±1.57a	1.71±0.40a
Mid	+oil	20.8±2.75bc	1.58±1.31b	0.32±0.34b
	-oil	25.7±2.90b	6.74±1.38a	1.33±0.35a
Distal	+oil	10.8±2.75c	1.71±1.31b	0.21±0.34b
	-oil	15.1±3.08c	2.40±1.46b	0.13±0.37b

Means in a column denoted by different letters are different ($P < 0.05$).

Table 5. Effect of dietary oil on performance of steers during backgrounding and finishing.

	Backgrounding			Finishing		
Parameter	- oil	+ oil	Improvement (%)	- oil	+ oil	Improvement (%)
ADG (kg/d)	1.06	1.23	16	1.20	1.32	11
DMI (kg/d)	7.62	6.53	-16	8.44	8.57	2
F:G	7.21	5.31	26	7.04	6.45	8

Steers were not implanted and were fed a diet containing pea hay, barley and soybean meal with (+ oil, at 6% of dry matter where the oil replaced part of the grain) or without oil (- oil).
ADG = average daily gain; DMI = dry matter intake; F:G = feed to gain ratio.

Table 6. Performance of finishing steers fed sunflower seed (SS) with (+) or without (-) silage either as whole seed (WSS) or as rolled seed (RSS).

	Dietary treatments				
Parameter	Control	9% RSS - Silage	9% RSS + Silage	14% WSS + Silage	14% RSS + Silage
Initial Wt., kg	484	485	489	481	482
Final Wt., kg	559	578	595	608	598
DMI, kg/d	7.42	7.37	8.19	8.55	8.35
ADG, kg/d	0.96	1.16	1.22	1.36	1.31
Feed/Gain	7.83	6.64	6.84	6.48	6.44
Carcass Characteristics					
Back fat, mm	10.7	11.7	11.3	13.7	13.7
Dressing %	58.3	58.3	59.0	59.7	59.1
Ribeye, cm^2	84.8	87.0	87.3	93.6	84.2
Quality grade (#A)	2.0	1.8	2.0	1.0	2.3
Liver Abscess					
Incidence %	33.3	30.0	27.3	36.4	33.3
Severity	2.3	1.0	1.0	0.0	2.5

Table 7. Growth characteristics of implanted steers fed diets without or with Mix-30™ .

Parameter	Dietary Treatment		S.E.	Probability of difference
	Control	Mix-30™		
Number of steers	121	121		
Initial weight (kg)	419	417	3.0	-
Final weight (kg)¶	567	577	3.7	0.061
Weight at shipment (kg)§	605	608	-	-
Average Daily Gain (kg)	1.8	1.9	0.04	0.073
Feed offered (tonne)¢	87.4	98.2	-	-
Dry matter intake (kg) includes Mix-30™	8.6	9.7	-	-
Blood urea nitrogen (mg/dl)	17.6	17.5	0.38	-

¶ 2000/06/08 was the last date the animals were individually weighed.
§ Weight at shipment 2000/07/05- animals were group weighed - no statistical evaluation.
¢ Feed for 121 steers in each group for 84 days from 03/16-06/08/00.
Mix-30™ - liquid byproduct from vegetable oil refining and corn syrup manufacture.

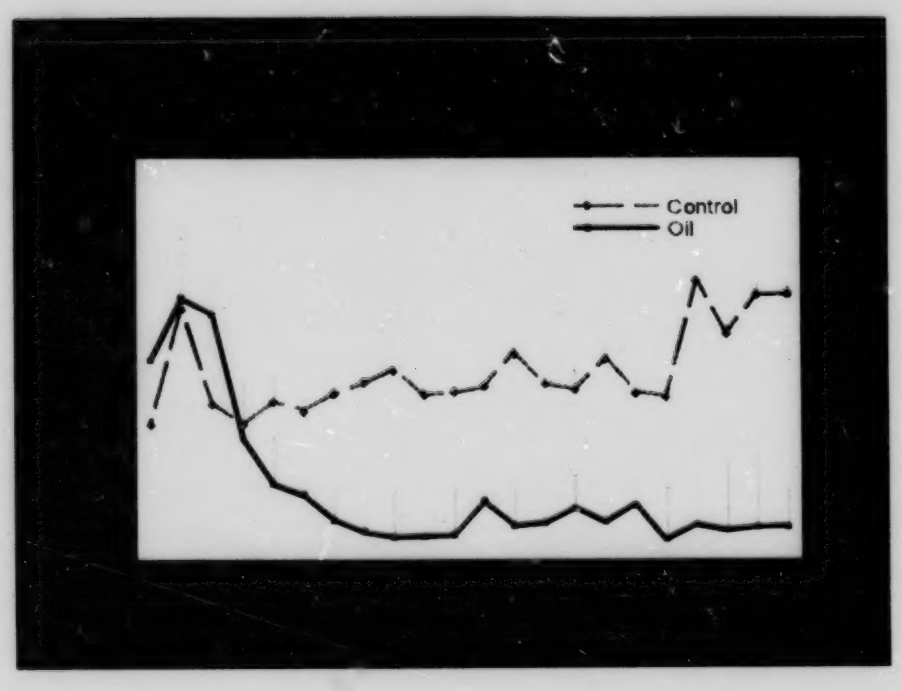

Figure 1. Effects of dietary supplementation with sunflower seed oil (6% of dietary dry matter) on ciliate protozoa concentration in rumen fluid of sheep. Vertical bars are standard error (n=5). The differences between treatment means were significant ($P < 0.05$) on days 6-49.

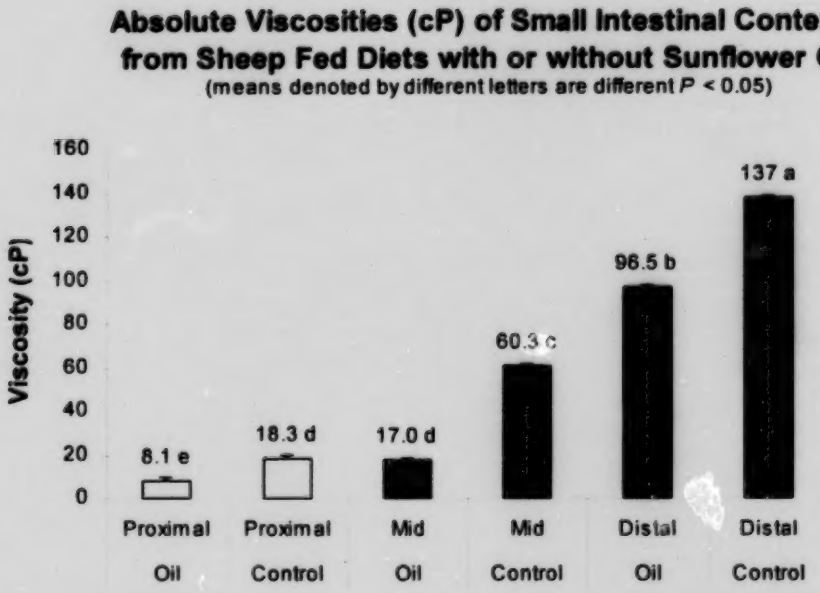

Figure 2. Absolute viscosities (cP) of small intestinal contents from proximal, mid and distal regions of sheep fed diets with or without sunflower oil. Means in a column denoted by different letters are different ($P < 0.05$).

Nutritional and Metabolic Disorders in Feedlot Cattle

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Key Points

- Digestive disorders account for approximately 25 to 33% of deaths in feedlot cattle, and likely contribute to decreased performance and efficiency of production.
- A variety of nutritional, management, genetic, behavioral, and environmental factors seem to be involved in the development of metabolic disorders in feedlot cattle.
- Excessive production of acid in the rumen is often either the cause of or a contributing factor to metabolic and nutritional disorders, including acute or sub-acute (chronic) acidosis, liver abscesses, feedlot bloat, and polioencephalomalacia.
- Foot rot and urolithiasis are two non-digestive disorders that seem to be affected by nutrition.

Introduction

Metabolic disorders in feedlot cattle are often complex disease conditions that typically occur at low frequencies. In a recent National Animal Health Monitoring System (NAHMS) survey (USDA, 2000b), 1.9% of cattle placed in feedlots developed digestive disease conditions, with a value of 1.1% for feedlots with a capacity of 1,000 to less than 8,000 animals to 2.0% for feedlots with a capacity greater than 8,000. The incidence of mortality from digestive diseases also is typically low. Smith (1998) reviewed three summaries of mortality data from large feedlots and indicated that mortality ranged from 0.17 to 0.419% of inventory per month; digestive disorders accounted for 30 to 42.4% of all mortality. Higher values were noted with Holstein cattle than with beef breeds (Smith, 1998), and digestive death rates seem to vary somewhat with feedlot size (Figure 1; Vogel and Parrott, 1994). In the NAHMS survey, the average cost of treating animals diagnosed with digestive problems was $6.19 U.S. (USDA, 2000b).

Both the complexity and low rate of occurrence of feedlot metabolic disorders has hampered research and diagnostic efforts. Galyean and Eng (1998) attempted to outline several factors and interrelationships among those factors that might affect metabolic disorders in feedlot cattle (Figure 2). Components of Figure 2 and interconnections among these components were not considered to be all-inclusive, but simply to consist of factors currently known or hypothesized to contribute to feedlot metabolic disorders. Galyean and Eng (1998) recognized that the role of some individual components (e.g., diet type and microbial population) is understood better than others (e.g., feeding and social behavior) and that potential interactions (connecting arrows in Figure 2) among these components often are poorly described. They concluded that a greater understanding of the etiology of metabolic disorders of feedlot cattle will require a multidisciplinary research approach, increased insight into various components of these disorders, definition of potential interactions, and recognition of currently unknown aspects of the problem.

This review will focus on describing the cause(s) of the most prevalent nutritional and metabolic disorders that occur in feedlot cattle, including acidosis, liver abscesses, bloat, and other disorders

like polioencephalomalacia, foot rot, and urinary calculi. Efforts will be made to identify areas in need of further research.

Acidosis

Importance and Etiology

Acidosis, often referred to as lactic acidosis, occurs as a practical consequence of the extensive use of highly processed grain and low levels of roughage in beef cattle finishing diets. In the NAHMS survey of nutritional and management practices in U.S. feedlots (USDA, 2000a), 61.3% of feedlots with a capacity of greater than 8,000 animals used steam-flaked (or steam-rolled) grain in finishing diets. Summaries of in vivo data clearly indicate that ruminal, intestinal, and total tract digestibility of starch is greater with steam-flaked corn and sorghum than with the dry-rolled counterparts (Huntington, 1997). Hence, processing cereal grains by heat and moisture methods (e.g., steam flaking) gelatinizes starch, increasing both the rate and extent of starch fermentation in the rumen, with concomitant acid production and increased risk for acidosis.

Owens et al. (1998) described both acute and sub-acute or chronic acidosis as conditions that can occur in cattle fed high-concentrate diets. Acute acidosis occurs as an overt illness in conjunction with consumption of an excessive quantity of readily fermented carbohydrate, which increases the total acid supply from the rumen (Owens et al., 1998). Lactic acid, normally found at very low concentrations in the rumen, can increase substantially, along with generally increased concentrations of volatile fatty acids (VFA). As a result of the increasing acid load, cattle with acute acidosis experience a drastic decrease in ruminal pH, with a pH of 5.2 considered as a benchmark (Owens et al., 1998). Absorption of acids into the blood steam can lead to systemic acidosis, stiffness in the legs, laminitis or founder (Nocek, 1997), severe ruminal lesions, and sometimes death. In contrast to acute acidosis, cattle with sub-acute acidosis do not typically show outward signs of illness but often exhibit decreased feed intake and performance. In sub-acute acidosis, ruminal pH drops substantially, with decreased feed intake following the acid load. A ruminal pH of 5.6 has been considered as a benchmark of sub-acute acidosis (Owens et al., 1998).

In an excellent review article, Owens et al. (1998) described the sequence of events leading to acidosis (Figure 3) and discussed potential points of control. The disease process begins when the animal consumes a large quantity of starch or other rapidly fermented carbohydrate. Glucose, normally found at extremely low concentrations in the rumen, is liberated from starch, resulting in ruminal glucose concentrations that can exceed those in the blood. High glucose concentrations can result in several negative consequences, including: 1) growth of organisms like Streptococcus bovis and other microbes that produce lactic acid; 2) growth of coliforms and amino-acid decarboxylating microbes that produce endotoxins and amines like histamine that can subsequently contribute to the development of laminitis (Nocek, 1997); and 3) increased ruminal osmolality, which aggravates ruminal acidity by inhibiting VFA absorption from the rumen (Owens et al., 1998). For reasons unknown at present, ruminal glucose concentrations remain high for an extended period during bouts of acute acidosis. Glucose is subsequently fermented via glycolysis through pyruvate to lactate or VFA. Ruminal microbes that ferment glucose to lactate tend to be fairly insensitive to low pH, but most lactate-utilizing microbes in the rumen are sensitive to low pH (Owens et al., 1998), thereby favoring production and accumulation of lactate. Lactate is produced as both the D and L isomers, with concentrations of greater than 40 mM considered reflective of severe acute acidosis (Owens et al., 1998). Total VFA concentrations increase along with lactate, and ruminal pH decreases substantially as absorption of acids is slowed by increased ruminal osmolality. As pH continues to drop and acid concentrations increase, absorption proceeds by passive diffusion. The capacity of the ruminal wall and liver to metabolize acids can be overwhelmed, leading to high blood concentrations

of acids that can potentially overpower the bicarbonate buffering system. In addition, blood osmolality increases because increased ruminal osmolality pulls fluid from the blood into the rumen and because acids that have accumulated in the blood have a direct effect on osmolality. If the animal is unable to restore homeostasis, death can result; however, even when animals recover, damage to the ruminal epithelium, laminitis, and lowered or erratic feed intake could have significant long-term effects on productivity.

Factors Affecting Acidosis

In commercial feedlots, cattle are adapted gradually to a final high-grain diet. This adaptation process is designed to minimize the risk of acidosis. Nonetheless, because pens of cattle are fed in commercial feedlots, with wide swings in intake by individuals within the pen possible, this transition period represents the time during which acidosis is most likely to occur. Roughages typically have a lower fermentable carbohydrate content than grains do, so increasing roughage decreases the likelihood for an acute ruminal acid load. Inherent buffering capacity of roughage and the ability of roughage to stimulate chewing and saliva flow also should aid in moderating ruminal pH. Sources of fiber other than those provided by traditional roughages might have beneficial effects. For example, based on results with individually fed cattle used to measure variation in feed intake and on ruminally cannulated cattle exposed to an acidosis challenge model, Krehbiel et al. (1995) concluded that a digestible fiber source like wet corn gluten feed did not eliminate acidosis but decreased the time that cattle were exposed to an acid insult. Careful bunk management that is designed to provide cattle with relatively constant day-to-day feed intake might be important in preventing acute and sub-acute acidosis, but with large numbers of animals per pen, individual animals might still overeat.

Although we have a reasonably complete understanding of the sequence of events leading to acute acidosis, our understanding of sub-acute acidosis is far from complete. Low or erratic feed intake and decreased gain are often associated with sub-acute acidosis, but results of production-level research to verify these effects are limited. Feed bunk management is an important aspect of nutritional consulting (Galyean, 1996); however, the extent to which feeding management systems affect the incidence of metabolic disorders like acidosis is largely unknown. Research is needed to identify the relationships between feeding management practices (e.g., restricted feeding, programmed feeding, and clean bunk management) and the incidence of acidosis. Variation in feed intake has been suggested to increase the incidence of metabolic disorders like acidosis (Bauer et al., 1995), and Galyean et al. (1992) found that varying intake decreased performance by feedlot cattle. Nonetheless, Zinn (1994) reported no effects, or even slight positive effects, of variation in intake on feedlot cattle performance. Galyean and Eng (1998) suggested that given the complexity of metabolic disorders like acidosis, a comprehensive evaluation of the relationship of feed intake variation to performance and the incidence of acidosis and other metabolic disorders is needed.

Feeding behavior might affect overeating and the potential for acidosis, but the extent of its impact is largely a matter of speculation because little research has been done in this area. Because feedlot cattle are typically fed in large groups, social behavior patterns (e.g., dominance structure in a pen of cattle, role models, and so on) might affect eating patterns and the incidence of metabolic disorders like acidosis. Research is needed to establish baseline data on the feeding and social behaviors of beef cattle fed high-concentrate diets, with studies conducted under commercial feedlot conditions in large pens likely being the most useful. The effects of previous nutritional history on feeding behaviors, and potential interactions of feeding behavior with nutrition and management need to be evaluated. If feeding and social behavior patterns affect the incidence of metabolic disorders, quantifying their effects should allow development of more effective model systems that would allow the use of individual animals to imitate metabolic disorders in feedlot cattle.

Feed additives can be an important tool for managing acidosis. Monensin has been shown to decrease day-to-day variation in feed intake (Stock et al., 1995), which coupled with a potentially greater frequency of smaller meals, a lower total feed intake, and direct effects on inhibition of lactate-producing bacteria (Coe et al., 1996; Owens et al., 1998) might decrease the likelihood of high acid production in the rumen. Ionophores like lasalocid and laidlomycin propionate do not seem to have major effects on feed intake (NRC, 1996), and their effect on acidosis and other metabolic disorders is not as well documented as with monensin. The role that dietary buffers play in feedlot acidosis is not well defined, nor is the role of dietary cation-anion difference (DCAD; Galyean and Eng, 1998). Further research in these areas is needed.

Liver Abscesses

Importance and Etiology

Liver abscesses are a relatively common disorder in feedlot cattle in the U.S., with occurrence rates ranging from 0 to nearly 100% (Nagaraja and Chengappa, 1998). Liver abscesses accounted for 46% of liver condemnations in the U.S., which was more than double the rate for liver flukes (22%), the second highest cause of condemnation (Nagaraja and Chengappa, 1998). The occurrence of liver abscesses is generally considered to be closely related to ruminal acidosis, as it is thought that a continual high acid load in the rumen can lead to parakeratosis or other types of insults to the ruminal wall, which in turn leads to clumping and necrosis of ruminal papillae (Ørskov, 1986). These ulcerative lesions, along with hairs or other foreign objects that become embedded in the ruminal epithelium, provide routes of entry into the portal system for microbes that cause liver abscesses; however, the exact mechanism of pathogenesis is unknown (Nagaraja and Chengappa, 1998). The incidence of liver abscesses can be high in certain geographical regions of the U.S., especially the Midwest feeding belt and northern Great Plains region. Affected livers are condemned at slaughter, which represents a loss of carcass weight of approximately 2% (Nagaraja and Chengappa, 1998). Cattle with severely abscessed livers (one or more large, active abscesses) gain less and are less efficient than cattle with no abscesses, but performance by cattle with less severely abscessed livers is not affected greatly (Brink et al., 1990; Table 1).

Fusobacterium necrophorum is the predominant organism cultured from liver abscesses in cattle, with Actinomyces pyogenes being a distant second among isolated pathogens (Nagaraja and Chengappa, 1998). F. necrophorum is a Gram-negative, anaerobic rod that is a normal inhabitant of the rumen, found both in the ruminal contents and associated with the ruminal wall (Nagaraja and Chengappa, 1998). It uses lactate as a substrate (Nagaraja and Chengappa, 1998), which would coincide with its presence in the rumen of cattle fed high-grain diets and potential links with acidosis. F. necrophorum produces several toxins and virulence factors that have been described in detail by Nagaraja and Chengappa (1998).

Controlling Liver Abscesses

Liver abscesses are visible at slaughter, and as noted previously, only severe abscesses affect animal performance; hence, detection of feedlot cattle with abscesses is virtually impossible. Ultrasonography has proven useful for monitoring experimentally induced abscesses, but its value in practice has been deemed negligible (Nagaraja and Chengappa, 1998). As a result of the inability to detect animals with liver abscesses, prevention is the only reasonable approach to use in practice.

Increasing dietary roughage level decreases the quantity of fermentable starch in the diet, thereby decreasing the acid load in the rumen, which should decrease the incidence of liver abscesses. Although some studies have shown a relationship between roughage level and the incidence of liver

abscesses (Gill et al., 1979; Zinn and Plascencia, 1996), others have not (Kreikemeier et al., 1990). In addition, the practical potential to increase roughage levels in feedlot diets is small given the advantage in cost per unit energy for grains vs roughages. Thus, careful adaptation of cattle from high-roughage to high-grain diets should be practiced to lessen the risk of acidosis and liver abscesses, but the role of adaptation or step-up programs and feed bunk management in the development of liver abscesses has not been extensively studied.

The addition of antibiotics to the feed is the primary means of controlling liver abscesses in feedlot cattle. In the U.S., bacitracin methylene disalicylate, chlortetracycline, oxytetracycline, tylosin, and virginiamycin are approved for prevention of liver abscesses (Nagaraja and Chengappa, 1998), with tylosin (8.8 to 11 mg/kg of diet or 60 to 90 mg/animal daily) being the most effective and most commonly used of these antibiotics. Decreases in liver abscess rates in the order of 75% have been reported in commercial-scale studies with tylosin (Nagaraja and Chengappa, 1998). Efforts to develop vaccines as a means of controlling liver abscesses have been less successful than using antibiotics like tylosin (Nagaraja and Chengappa, 1998).

Feedlot Bloat

Importance and Etiology

Bloat is frequently reported as a cause of death in feedlot cattle, and has been recognized as an important component of mortality associated with digestive disorders (Nagaraja et. al., 1998). Bloat occurs when the animal is prevented from expelling ruminal gas, which subsequently places pressure on the diaphragm and lungs, thereby affecting breathing and potentially resulting in death. Feedlot bloat is characterized as frothy, with the formation of stable foam. Eructation via the esophagus is inhibited when the cardia is covered with foam (Cheng et al., 1998). Free-gas bloat, which usually occurs as a result of physical obstruction or damage to the cardia or esophagus, or as a result of decreased ruminal motility, occurs in feedlot cattle, but frothy bloat is a more common cause of bloat in feedlot cattle (Cheng et al., 1998). Feedlot (frothy) bloat often occurs during adaptation to high-grain diets (Nagaraja et al., 1998), but sporadic outbreaks are frequently reported in cattle that are well adapted to a finishing diet. These "bloat blizzards" as they are sometimes called by disgusted nutritionists and feedlot managers are typically of limited duration and almost always of unknown cause(s).

With pasture (e.g., alfalfa or annual winter wheat) frothy bloat, high soluble protein levels in the forage, coupled with rapid fermentation of the forage and possible animal factors are thought to contribute to the formation of stable foam. With highly fermentable grain-based diets, however, microbial factors seem to be responsible for the formation of stable, frothy contents (Nagaraja et al., 1998). Production of mucopolysaccharide slime by ruminal bacteria, coupled with release of undefined macromolecules when bacterial cells are lysed, increases ruminal fluid viscosity and contributes to development of stable foam (Cheng et al., 1998). Streptococcus bovis is known to produce slime, and increased numbers of S. bovis have been reported with bloat (Cheng et al., 1998); however, other bacteria might be involved, and the microbial changes that lead to and are associated with feedlot bloat need to be described more completely.

Factors Affecting Feedlot Bloat

As with acidosis and liver abscesses, feedlot bloat is largely a disease that results from the feeding of high-grain diets. Hence, factors associated with the feeding of grains will affect the incidence and severity of bloat. Because cereal grains differ in the rate and extent of fermentation of starch, differences exist in the rate of acid and gas production among grains. Among cereal grains,

the ranking of ruminal and total tract starch digestion is oats = wheat > barley > corn > sorghum (Huntington, 1997). Differences in the nature of endosperm protein also exist among grains, which are associated with differences in ruminal starch digestion. Theurer (1986) indicated that the total tract protein digestibility of corn, sorghum, and barley was inversely related to ruminal and total tract starch digestion. Wheat is often considered the most bloat-provocative grain in feedlot diets (Cheng et al., 1998). Indeed, wheat is rarely fed as the sole dietary grain, typically being mixed with corn or sorghum in practical feedlot diets.

Grain processing increases rate and extent of starch digestion, thereby potentially affecting the incidence of bloat. Fine particle size seems to promote frothiness, perhaps through accelerated production of bacterial slime (Cheng et al., 1998), and smaller particles of both gelatinized and ungelatinized grain are digested at a faster rate than larger particles are (Galyean et al., 1981), leading to higher gas production. Care should be taken in processing and milling of grains to avoid creation of fine particles. Responses to processing methods like steam flaking are sufficiently large (Owens et al., 1997) that feeding less extensively processed grain to avoid feedlot bloat is probably not a viable alternative.

As with acidosis and liver abscesses, adding roughage to the diet should slow the overall rate of fermentation, stimulate salivation, and lower acid production, thereby decreasing the likelihood of bloat. Feedlot bloat often occurs as cattle are transitioned from high-roughage, starter diets to high-grain, low-roughage finishing diets (Cheng et al., 1998). Careful, step-wise transitions should aid in decreasing the incidence of bloat, and higher roughage levels in finishing diets should prove beneficial. Increasing the level of roughage beyond a certain point, however, is not economically feasible because of the high cost of energy from roughages vs grains and the potential for bulk to limit energy intake. In commercial feedlots, roughage level is sometimes increased temporarily as a means of bloat control at times when the incidence of feedlot bloat is extremely high. More attention should be given to the effect of different roughage sources on feedlot bloat, but at present, there is no basis for choosing among roughages with respect to their ability to decrease feedlot bloat. The consistency of roughages, even within the same roughage type, further decreases the potential to use roughage source and level as a routine means of managing the incidence of feedlot bloat.

Ionophores, most notably monensin, seem to be useful for decreasing the incidence of feedlot bloat. Feeding monensin decreases the incidence of frothy bloat on irrigated wheat pasture (Branine and Galyean, 1990). These compounds inhibit many of the Gram-positive bacteria that produce lactate and mucopolysaccharide slime (Cheng et al., 1998). In addition, effects of monensin on eating patterns and day-to-day variation in feed intake (Stock et al., 1995) of cattle might be beneficial for decreasing feedlot bloat. If the benefits of monensin on feedlot bloat occur totally through effects on feed intake, restricting intake might be equally beneficial, but this has not been tested in a research setting. Poloxalene, an antifoaming agent, is very effective for control of pasture bloat; however, the incidence of feedlot bloat is decreased but not eliminated by poloxalene (Cheng et al., 1998). Animal susceptibility to bloat varies, presumably as a result of differences in anatomy, ruminal motility, eating rate, production of saliva, and other unknown factors (Nagaraja et al., 1998).

Other Metabolic Disorders

Polioencephalomalacia

Outbreaks of polioencephalomalacia (PEM), a neurological disorder of ruminants, occur sporadically in feedlots. Clinically, the disease is characterized by blindness, incoordination, muscle tremors, and possible recumbency with seizures (Blood and Radostits, 1989; Gould, 1998). As noted by Gould (1998), the term PEM is used in two ways: 1) to describe a specific disorder caused by

altered thiamine status, and 2) to describe a specific brain lesion that can result from various causes. Indeed, characteristic brain lesions are evident at post-mortem examination (Blood and Radostits, 1989), and cerebrocortical necrosis is synonymous with PEM (Gould, 1998).

The role of thiamine in PEM has been studied for many years; however, questions remain as to exactly how thiamine is involved in PEM. Animals treated at the onset of PEM signs with intravenous thiamin hydrochloride often respond and recover. Thiamin deficiency induced by production of thiaminase or thiamine anti-metabolites in the rumen has been implicated (Blood and Radostits, 1989). Although decreased concentrations of thiamine have been noted in some studies, others have shown no relationship between tissue and ruminal fluid thiamine concentrations and PEM, and severe thiamine deficiency has not induced PEM (Gould, 1998). Lead poisoning also results in brain lesions characteristic of PEM, with diagnosis of lead poisoning dependent on evaluation of blood and tissue lead concentrations (Gould, 1998).

Recent research (Gould, 1998) indicates that PEM can be caused by high levels of sulfur in the water or feed, with subsequent over-production of hydrogen sulfide (H2S) in the rumen, accumulation of H2S in the ruminal gas cap, and absorption into the blood stream. Sulfur intake from all sources, including water, should be considered, with the maximum tolerable level of 0.4% of dietary DM (NRC, 1996) used as a guideline. High intakes of sulfates seem to enhance the ability of ruminal microbes to produce H2S, and consideration should be given to dietary S-containing amino acids as contributors to the H2S pool (Gould, 1998). Acidic conditions in the rumen favor higher concentrations of H2S in the gas cap (Gould, 1998), so factors affecting ruminal acidity discussed previously in the section on acidosis are likely relevant to the development of PEM. Further research is needed to more clearly define the dietary and management conditions that lead to sulfate-related PEM, but, at present, decreasing total sulfate intake (feed and water) is the only means of dealing with the problem.

Foot Rot

Foot rot, or necrotic pododermatitis, is a contagious disease that causes inflammation of the foot and lameness (Blood and Radostits, 1989). Fusobacterium necrophorum and Bacteroides melaninogenicus are the primary bacteria that are isolated in cases of foot rot (Griffin, 1998). Foot rot is more common in wet and humid weather and when surface conditions in the feedlot are wet or muddy. Antibiotic therapy and treatment of the foot lesions are the usual course of action for foot rot, and prevention typically involves maintaining pens in a manner that will decrease wet, muddy conditions. Nutritional factors might be important in foot rot, but further research is needed. Supplementation of pasture cattle with zinc methionine decreased the incidence of foot rot (Brazle, 1994), and anecdotal evidence suggests that Zn supplemented at higher than NRC (1996) recommended levels and(or) organic complexes of Zn might be useful in feedlots that frequently experience foot rot problems. The findings of a recent study with sheep, however, indicated that feeding 2 g of ZnSO4.7H2O twice weekly over a 4-mo period failed to control foot rot (Rejas Lopez et al., 1999). Ethylenediamine dihydroiodide (EDDI) fed to provide 50 mg or more per animal daily also has been used as a preventative measure, but over-consumption can lead to potential toxic effects (Griffin, 1998). Current U.S. Food and Drug Administration (FDA) regulations limit the feeding of EDDI to 10 mg per animal daily as a source of iodine, a level that is not likely to have beneficial effects on foot rot (Apley and Fajt, 1998).

Urinary Calculi

The blockage of the urinary tract by small stones or calculi results in a condition known as urinary calculi or urolithiasis, which is often referred to by the common term "water belly." Small

stones in the kidney seem to have limited consequences; however, movement of the stones to the ureters or urethra can result in urinary blockage (Emerick, 1988). Two different forms of urolithiasis occur, phosphatic or siliceous. In feedlot cattle, phosphatic calculi, which are comprised of Ca and Mg phosphates, are the most common, with siliceous calculi typically occurring in grazing cattle (Emerick, 1988). Urolithiasis is only important with steers because the urinary tract is physically more likely to be obstructed than with heifers. Initial symptoms in affected animals include frequent attempts to urinate, and the tail sometimes seems to be pumping or twitching. As the condition advances, affected steers will often kick at their abdomen, stamp their feet, and repeatedly stand up and lie down (Griffin, 1998). If the calculi are not removed, the urethra or bladder ruptures, ultimately leading to death of the animal. Surgical intervention is almost always required, and carcasses from affected animals are typically condemned, restricting the possibility of salvage before resolution of the disease (Griffin, 1998).

Phosphatic calculi contain an organic matrix, largely composed of proteins, in addition to Ca and Mg phosphate, but the role of protein in calculi formation seems limited (Emerick, 1988). Circumstances that lead to the production of alkaline urine, along with high dietary concentrations of P are conducive to development of phosphatic urolithiasis (Emerick, 1988). Dietary concentrations of P that are approximately twice the recommended concentrations increase the incidence of urolithiasis by approximately 50%, and the Ca:P ratio seems to be and important factor (Emerick, 1988). Low urine volume also can contribute to an increased incidence of urolithiasis, which might account for a higher incidence of the disease during the winter when water intakes would be lower than in warmer seasons (Emerick, 1988).

Prevention of phosphatic urinary calculi revolves around keeping dietary Ca and P concentrations in line with recommendations (NRC, 1996), and a Ca:P ratio in the range of 1.5:1 to 2:1 is advisable (Emerick, 1988). Adding urine acidifiers like NH4Cl or CaCl2 to the diet is useful for prevention of urinary calculi. According to Emerick (1988), NH4Cl fed to provide 28.4 to 42.5 g/animal daily to fattening cattle is approved by the FDA for prevention of urinary calculi. Emerick (1988) also noted that including CaCl2 at approximately 1% of the diet has been reported to decrease the incidence of phosphatic urinary calculi. Using DCAD values might be a useful means of tracking potential dietary effects on urine pH (NRC, 2001), but practical data to assess the utility of DCAD values for this purpose are not available. Increasing urine volume by adding salt (NaCl) to the diet has been suggested as a means of decreasing the incidence of phosphatic urinary calculi; however, the concentrations of salt needed to achieve this effect are too high for long-term use (Emerick, 1988).

Implications

Metabolic and nutritional disorders are complex, likely involving nutritional, environmental, genetic, management, and animal behavior aspects. Ruminal acidity is often the culprit in metabolic disorders. Acidosis occurs with consumption of high levels of rapidly fermented grains, potentially leading to overproduction of VFA and lactate in the rumen. Liver abscesses can result from acid insults that erode the ruminal wall and allow bacteria to reach the liver where abscesses form. Feedlot bloat is most likely to occur with high-grain diets that promote high rates of gas production and the growth of microbes that produce mucopolysaccharide slime, leading to the formation of stable foam. Polioencephalomalacia is most frequently associated with feeding high-grain diets, and recent research has implicated the production of H2S in the rumen, with subsequent absorption into the blood stream, as a potentially important cause of PEM. Of non-digestive tract disorders that occur in feedlot cattle, foot rot and urinary calculi are two that seem to have links to nutrition.

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Table 1. Effects of liver abscesses on feedlot performance by beef cattlea

	Liver abscess severity scoreb			
Itemc	None	A-	A	A+
No. of steers	405	52	37	72
Days on feed	131 (0.8)	133 (2.1)	131 (2.1)	131 (1.4)
Initial BW, kg	305 (1.9)	320 (4.8)	316 (5.6)	311 (3.4)
Final BW, kg	470 (2.1)	484 (5.6)	477 (7.2)	461 (4.8)
Hot carcass wt., kg	289 (1.4)	298 (3.9)	293 (4.7)	276 (3.1)
ADG, kg/d	1.27 (0.01)	1.23 (0.04)	1.24 (0.04)	1.15 (0.03)
DMI, kg/d	8.39 (0.06)	8.27 (0.19)	8.42 (0.15)	7.96 (0.50)

aAdapted from Brink et al. (1990).
bA- = one to two small abscesses; A = two to four small, active abscesses; A+ = one or more large, active abscess.
cNumbers in parenthesis are standard errors. No differences were significant ($P < 0.05$) in the overall data set; however, significant differences were detected in subsets of the data. See Brink et al. (1990) for details.

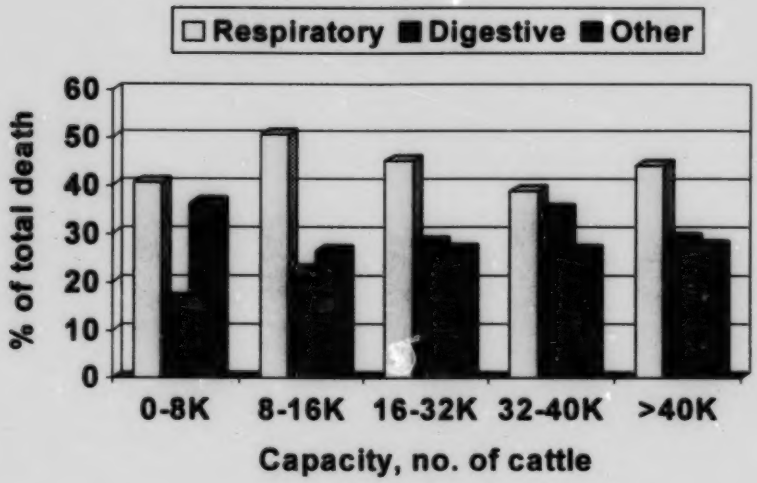

Figure 1. Metabolic death losses in feedlots of varying size. Adapted from Vogel and Parrott (1994). Mortality as a percentage of occupancy per month averaged 0.326, 0.323, 0.265, 0.239, and 0.204 for capacities of 0-8K, 8-16K, 16-32K, 32-40K, and >40K, respectively.

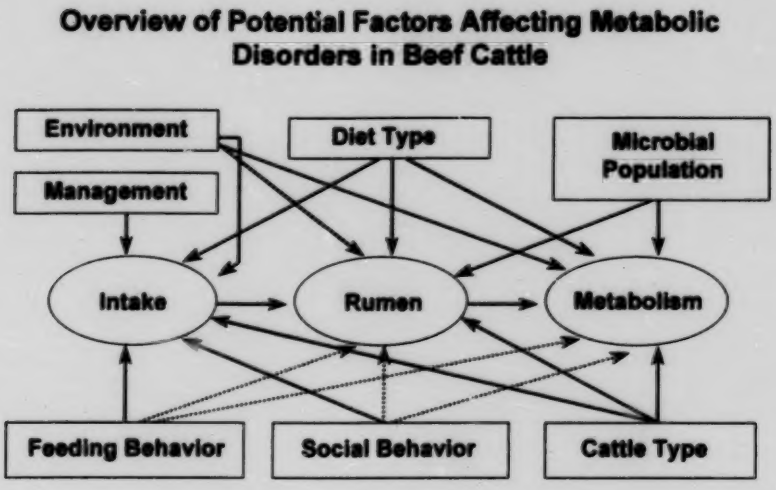

Figure 2. Possible factors and interrelationships among factors that affect metabolic disorders in feedlot cattle. Solid arrows signify relationships that are known to exist, whereas dotted arrows represent possible relationships. Adapted from Galyean and Eng (1998).

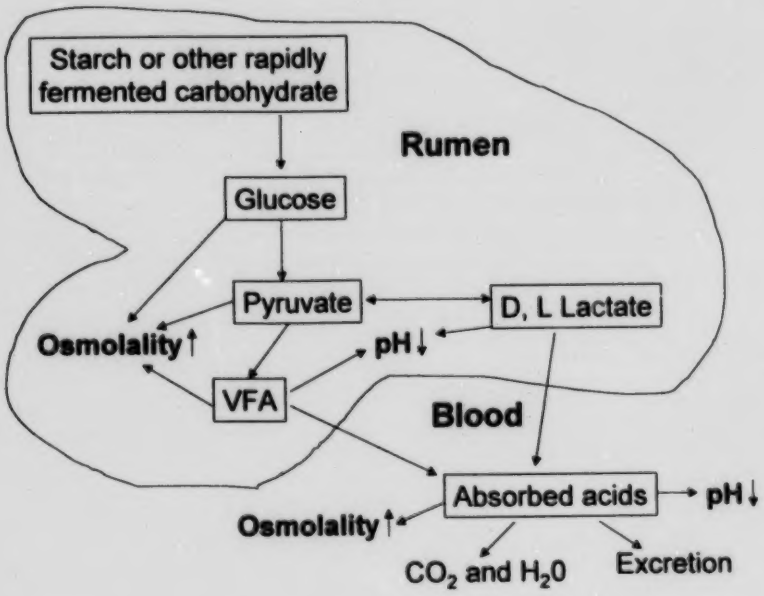

Figure 3. The etiology of acidosis in cattle. Adapted from Owens et al. (1998).

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Net Feed Intake in Beef Cattle

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Key Points

- Net or residual feed intake is a measure of an animal's energy requirements for maintenance and is moderately heritable.
- Large variation in net feed intake exists within and among breeds or biological types.
- Net feed intake is independent of body size, growth and composition of gain.
- Animals with low net feed intake have lower Metabolizable Energy (ME) intake and heat production than animals with high net feed intake, but may be similar in growth, carcass traits and body composition.
- Differences in net feed intake are likely due to differences in ME intake and metabolic processes.
- Animals with low net feed intake emit less methane than animals with high net feed intake.
- Seedstock producers must exploit net feed intake in an effort to improve feed efficiency and to remain globally competitive.

Introduction

Feed efficiency has a tremendous influence on the unit costs of production, and thus on the competitiveness of meat products in both domestic and international markets. In beef production, only 5% of the total life cycle dietary energy expended is used for protein deposition, whereas, pork and poultry are more efficient at 14 and 22%, respectively. Major reasons for the inefficiency of beef production include their relatively low and slow reproductive rates and the high energetic cost of maintaining the animal. It has been estimated that 71% of the total dietary energy cost in beef production is used for maintenance, including maintenance of the dam. Seventy to 75% of the total annual energy requirement for a beef cow is used for maintenance regardless of cow type (Ferrell and Jenkins, 1985).

Genetic variation for maintenance energy requirements of cattle is moderate to high, suggesting an opportunity to select for more efficient cattle (DiConstanzo et al., 1990). Selection for lower maintenance requirements is difficult and most measures of feed efficiency are related to each other and to measures of body size and growth rate (Arthur et al., 2001b). Net feed intake (NFI) or

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residual feed intake is defined as the difference between an animal's actual feed intake and its expected feed requirements for maintenance and growth over a specific test period. It is independent of body size and growth and has been identified as a trait that reflects the maintenance requirements of individual animals (Archer et al., 1998). However, differences in efficiencies of growth may also be due to differences in composition of live weight gain. For example, differences in rates of water and protein accretion have an influence on rate and efficiency of body weight gain, primarily because of the lower energy content of water and protein relative to fat (Ferrell and Jenkins, 1998). Conversely, higher maintenance costs are frequently more highly associated with greater visceral organ weights and increased feed intake (Ferrell and Jenkins, 1998). In addition, higher maintenance costs are more associated with body protein than with body fat (Pullar and Webster, 1977).

The objective of this paper is to discuss the merits of using net feed intake as a measure of animal maintenance requirements for energy and to discuss new research which characterizes the growth, carcass characteristics, body composition and energetics of animals with low, medium and high net feed intake.

Net Feed Intake

The Potential

Researchers at the Trangie Agricultural Research Centre in Australia have evaluated 1166 calves and 116 sires for post-weaning net feed intake (Archer at al., 1998). Breeding bulls were identified which consumed 300 kg less feed over a 120-day test period to maintain a similar live-weight and achieve the same growth performance. Similar results have been reported by Canadian researchers at the Lacombe Research Centre (Basarab et al., 2001a). In the first year of a two year serial slaughter study, 75 steers from five genetic strains were monitored for individual animal feed intake over a finishing period ranging from 71 to 183 days. Steers grew at 1.46 kg/day, had an actual feed intake of 10.9 kg/day and a net feed intake of 0.00 (SD=0.66) kg/day. Individual animals varied in net feed intake from efficient (-1.49 kg/day) to inefficient (+1.54 kg/day) (Figure 1). This variation in net feed intake represented a difference of more than $36 in feed costs (assuming feed costs of $0.101/kg as fed) during a 120-day test period or approximately $263 million annually for Alberta's feeder cattle industry.

The second point illustrated by Figure 1 is that net feed intake is an indicator of a trait that is different from feed to gain ratio, a traditional measure of feed efficiency. While net feed intake was positively correlated ($r = 0.32$, $P < 0.01$) with feed to gain ratio, many animals with low net feed intake ratios had high feed to gain ratios and some animals with vastly different net feed intakes had similar feed to gain ratios. For example, steers A, B and C were similar in feed to gain ratio (8.2 to 8.4:1) and average daily gain, but vastly different in net feed intake which ranged from -1.26 to +1.26 kg/day. The reason for this difference appears to be related to actual feed or metabolizable energy (ME) intake, with steers that had low ME intakes tending to have lower net feed intakes ($r = 0.51$, $P < 0.0001$). These results are consistent with Ferrell and Jenkins (1998), who showed that the efficiency of ME use for retained energy was not constant, but decreased as ME intake increased. They suggested that a portion of nonlinearity in the relationship between retained energy and ME intake was due to a depression in metabolizability of the diet at high levels of intake, higher maintenance cost or heat increment of feeding at higher levels of feed intake and heavier visceral organ weights, including weights of stomach complex, intestines, heart, lung, kidney and spleen. Thus, net feed intake may be an indicator of the animal's maintenance requirements rather than of growth, size and/or appetite.

The Problem

In an Australian study, researchers mated the top 5% of efficient bulls (negative net feed intake) to the top 50% of efficient heifers, while the bottom 5% of inefficient bulls (positive net feed intake) were mated to the bottom 50% of inefficient heifers (Arthur et al., 2001a). The results revealed that after two generations of selection for net feed intake, the progeny from efficient parents had lower net feed intake, actual feed intake and feed conversion ratio than progeny of inefficient parents (Table 1). There was no correlated response in either yearling weight or average daily gain. However, Richardson et al. (2001) reported that the efficient progeny had 12.3% less carcass fat than inefficient progeny (9.9 vs 11.3 % of final live weight, $P < 0.05$). This lower carcass fat content in the efficient progeny raises several concerns, such as the potential genetic antagonisms of net feed intake with marbling and reproductive fitness and the effect that composition of gain has on the true energetic efficiency of the animal. Thus it is possible that differences in net feed intake were partially due to differences in fattening and not due to inherent differences in the energy required for maintenance and growth of specific animal types. This is a key factor in understanding net feed intake, as deposition of fat in the body requires up to 10 times as much feed energy as depositing the same weight of non fat material (protein, carbohydrate and mineral material). Chemical fat is found throughout the body in all organs and tissues, mainly in the form of stored triglycerides, but also in structural lipids such as phospholipids and cholesterol in every cell membrane. In order to account for energy conversions (and hence feed efficiency), it is essential to quantify the amount of these high energy lipid substances being deposited in each unit of live weight gain.

Body composition and net feed intake

Preliminary results from a serial slaughter study conducted at the Lacombe Research Centre provides new insight into the relationship between body composition and net feed intake. Basarab et al. (2001a), in cooperation with BeefBooster Cattle Alberta Ltd., measured the individual feed intake of 90 steers (331 kg; SD=40 kg) over a finishing period ranging from 71 to 183 days. Eighteen steers from each of the five BeefBooster strains (M1, M2, M3, M4 and TX) were represented and all animals were adjusted to a high-barley diet. Three steers from each genetic strain were selected at random on days 1, 71, 99, 127, 155 and 183 and processed at the Lacombe Research Centre abattoir. Carcass, organ and tissue weights were obtained and body composition evaluated. No relationships were observed between net feed intake and any of the simple measures of body composition, such as final body condition score ($r = -0.09$, $P = 0.42$), final ultrasound backfat thickness ($r = 0.00$, $P = 0.97$), carcass backfat thickness ($r = 0.07$, $P = 0.57$), carcass marbling ($r = -0.04$, $P = 0.74$) or empty body fat ($r = -0.01$, $P = 0.96$). In addition, no relationships were observed between net feed intake and gain in empty body fat (Figure 2) or net feed intake and gain in empty body protein (Figure 3). This result is partially explained by the strong positive relationship between metabolic mid-weight and gain in empty body fat content ($r = 0.80$, $P < 0.0001$), which automatically adjusts for changes in empty body fat content. Thus preliminary results indicate that net feed intake need not be adjusted for empty body fat content as long as the feed intake test period is conducted when the animals have between 2 mm and 18 mm of ultrasound backfat thickness or 13% to 34% empty body fat.

Characterization of animals with different net feed intakes

Researchers from both Australia and Canada have shown that animals with low net feed intake consume less dry matter and have a lower feed conversion ratio than animals with average or high net feed intakes (Arthur et al., 2001a; Basarab et al., 2001b). In both studies, growth rates and body size

among animals with different net feed intakes were similar. The Canadian study also revealed for the first time that animals with different net feed intakes were similar in carcass characteristics (Table 2), empty body weight, relative proportions of most organs and tissues in empty body weight (Table 3), empty body composition, accretion rates of protein, fat and energy and in energy retained (Table 4). In this study, the major difference among net feed intake groups was in heat production, with low net feed intake steers having an 11.2% lower heat production value than medium net feed intake steers and 17.0% lower than high net feed intake steers (Table 4). These results are consistent with Herd and Bishop (2000), who reported a positive genetic correlation between maintenance energy costs and net feed intake in young Hereford cattle indicating that high net feed intake cattle have higher maintenance energy costs than low net feed intake cattle. These higher maintenance energy costs may be attributed to a depression in metabolizability of the diet at high levels of intake, higher maintenance cost or heat increment associated with higher levels of feed intake and heavier visceral organ weights (Ferrell and Jenkins, 1998). Thus, maximum efficiency of growth may occur at less than maximum intakes.

Net feed intake and methane production

Methane emissions from cattle range from 2 to 12% of gross energy intake. This represents not only a substantial loss in efficiency of production, but the methane emitted contributes to greenhouse gases. Cattle with negative net feed intakes may produce less methane and manure than cattle with positive net feed intakes. Okine and co-workers (Okine et al., 2001) recently calculated the methane emission and manure production from feeder steers with low, medium and high net feed intakes. They found no difference in methane emission as a percent of gross energy ($P > 0.05$) among animals with different net feed intakes (Table 5). However, daily methane production, either expressed as g/day or as MJ/day, was lower ($P < 0.05$) for steers with low net feed intake than steers with high net feed intake. In addition, yearly methane emissions from low net feed intake steers was 21% lower ($P = 0.02$) than for high net feed intake steers (56.6 vs 68.5 kg/year). Yearly manure, N, P and K production were also 14.5, 16.9, 17.0 and 17.1% lower for low net feed intake than for high net feed intake steers. Steers with low net feed intakes and with above average daily gain (1.46 kg/day) produced 45.9% less ($P < 0.05$) methane than steers with high net feed intake and below average daily gain values (52.5 vs 76.6 kg/year; Figure 4). Assuming that a carbon credit is worth $7.50 CAN/tonne of carbon dioxide equivalence, the methane credit for the low net feed intake steers with above average daily gain would be $3.80 CAN/ head/yr relative to the high net feed intake steers with below average daily gain.

Implications

Cattle with low net feed intake (efficient) consumed less feed and had lower heat production than those with average or high net feed intake (inefficient). In addition, low net feed intake animals were similar in growth, carcass characteristics, body composition and accretion rates of protein, fat and energy. Net feed intake or residual feed intake can be used to identify animals with lower energy requirements for maintenance and is independent of growth, size and body composition. In addition, selection for net feed intake has the potential to reduce methane emissions from cattle and, possibly, result in new agriculture investment due to methane credits sold to the energy sector.

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Table 1. Performance of progeny from low or high net feed intake bulls and heifers after five years of selection[a]

Traits	Low net feed intake parents	High net feed intake parents	Yearly Correlated response
Number of animals	62	73	
Net feed intake, kg/day	-0.54 a	0.71 b	0.25
365 day live weight, kg	384.3	380.7	0.72
Average daily gain, kg/day	1.44	1.40	0.01
Actual feed intake, kg/day	9.4a	10.6b	0.24
Feed conversion ratio, kg/kg	6.6a	7.8b	0.24

[a] Adapted from Arthur et al. (2001a)
b,c means in the same row differ, $P < 0.05$

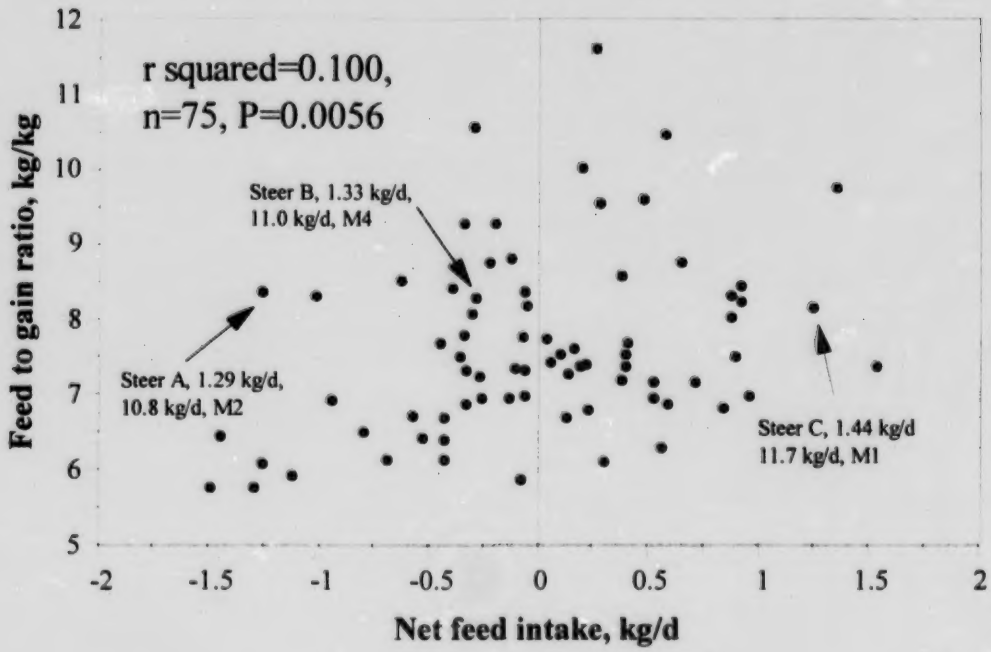


Figure 1. Relationship between feed to gain ratio and net feed intake.

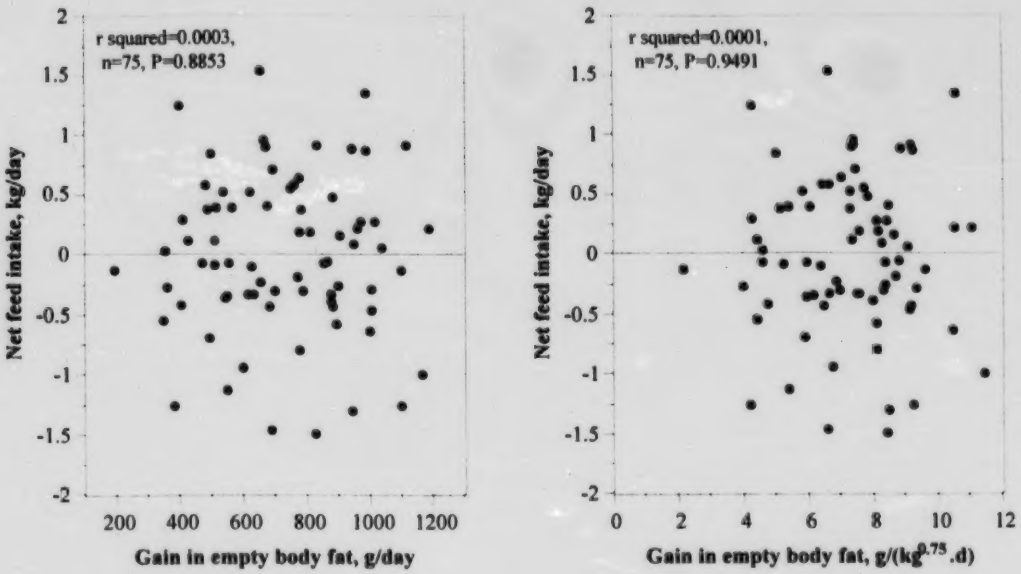


Figure 2. Relationship between net feed intake and gain in empty body fat of steers, expressed either in grams per day or grams per kilogram of metabolic weight per day.

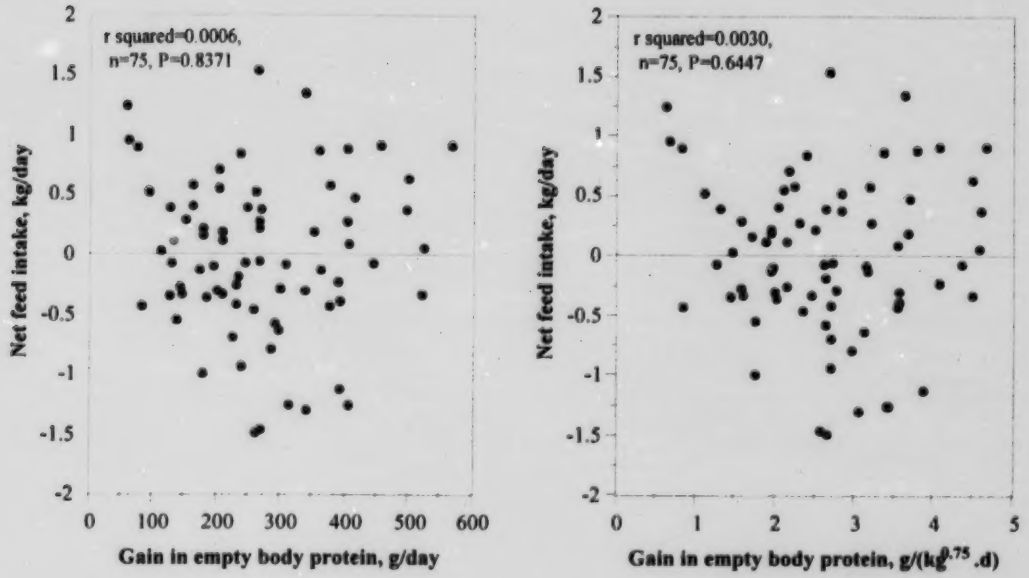


Figure 3. Relationship between net feed intake and gain in empty body protein in steers, expressed either in grams per day or grams per kilogram of metabolic weight per day.

Table 2. Feed intake, growth, feeding behaviour, and carcass characteristics of steers with high, medium and low net feed intakes.

Traits	Net feed intake group[z]			SEM	Probability
	High	Medium	Low		
Number of steers	23	31	21		
Net feed intake, kg/day	0.76 a	-0.04b	-0.70c	0.07	0.0001
Feed intake					
DM intake, kg/day	8.6a	8.2b	7.9c	0.08	0.0004
ME intake, MJ/day	94.7a	90.1b	87.2c	0.9	0.0004
Growth traits					–
Feed:gain ratio, kg DM/kg gain	6.20 a	5.67b	5.30b	0.13	0.0024
Metabolic mid-weight, kg^{75}	98.4	98.3	100.5	0.7	0.1201
Average daily gain, kg/day	1.41	1.48	1.52	0.04	0.2354
Feeding behaviours					
Feeding duration, min/day	85.6	77.5	84.2	2.8	0.1711
Feeding frequency, events/day	8.5	7.7	7.1	0.4	0.1768
Carcass traits					
Cold carcass weight, kg	287.6	287.7	294.0	3.1	0.4149
L. thoracis area, cm^2	73.9	76.5	78.7	1.5	0.2573
Grade fat, mm	10.0	9.0	9.2	0.6	0.5853
Marbling score[y]	441.5	446.2	449.5	11.5	0.9343

[z] Net feed intake groups are defined as follows: High, net feed intake is > 0.5 SD below the mean; Medium, net feed intake is ± 0.5 SD above and below the mean; Low, net feed intake is < 0.5 SD above the mean.

[y] Marbling score is a measure of intramuscular fat: trace marbling or less = 100 to 399 (Canada A quality grade); slight marbling = 400 to 499 (Canada AA quality grade); small to moderate marbling = 500 to 799 (Canada AAA quality grade); slightly abundant or more marbling = 800 to 1100 (Canada Prime).

a,b,c means in the same row differ, $P < 0.05$

DM = dry matter; ME = metabolizable energy

Table 3. Relative proportion of organs and tissues in empty body weight of steers with high, medium and low net feed intake.

Traits	Net feed intake group[z]			SEM	Probability
	High	Medium	Low		
Number of steers	23	31	21		
Empty body weight (EBW), kg	450.2	450.2	459.4	4.2	0.3330
Organs and tissues at slaughter, % of EBW					
Hide	8.54	8.50	8.75	0.16	0.5904
Blood	3.15	3.01	3.16	0.07	0.3605
Head+tongue+feet+tail	5.22	5.24	5.27	0.05	0.8804
Liver	1.45	1.41	1.42	0.03	0.6821
Empty stomach complex	4.50	4.13	4.17	0.11	0.1673
Empty small and large intestine	5.57	5.93	5.93	0.16	0.3845
Heart	0.49	0.50	0.50	0.01	0.8015
Lung and trachea	1.66	1.62	1.53	0.03	0.1129
Kidney	0.22	0.21	0.22	0.01	0.6727
Kidney fat	1.98	2.12	2.03	0.08	0.6054
Spleen	0.21	0.22	0.21	0.01	0.6623
Bladder	0.30	0.28	0.34	0.02	0.0781
Hanging tender	0.27	0.27	0.27	0.01	0.9790
Trim	1.74	1.70	1.66	0.04	0.6071
Reproductive tract	0.16	0.17	0.14	0.01	0.2035

[z] Net feed intake groups are defined as follows: High, net feed intake is > 0.5 SD below the mean; Medium, net feed intake is ± 0.5 SD above and below the mean; Low, net feed intake is < 0.5 SD above the mean.

a,b,c means in the same row differ, $P < 0.05$

Table 4. Body composition, daily accretion rates of protein, fat and energy, retained energy and heat production from steers with high, medium and low net feed intake.

Traits	Net feed intake group[z]			SEM	Probability
	High	Medium	Low		
Number of steers	23	31	21		
Empty body (EBW) composition					
Water, g/kg EBW	525	529	518	5.2	0.4032
Fat, g/kg EBW	269	268	277	6.0	0.6218
Protein, g/kg EBW	162	158	157	2.1	0.3646
Ash, g/kg EBW	44b	45b	48a	0.9	0.0249
Energy, MJ/kg EBW	14.3	14.2	14.5	0.2	0.6321
Accretion rates					
Protein, g/day	273	255	264	13	0.7250
Fat, g/day	723	713	760	31	0.6440
Energy, MJ/day	34.8	34.0	36.0	1.2	0.5716
Retained energy, KJ/(kg^{75} .d)	349	341	355	12	0.7703
Heat Production, KJ/(kg^{75} .d)	619a	579b	514c	13	0.0002

[z] Net feed intake groups are defined as follows: High, net feed intake is > 0.5 SD below the mean; Medium, net feed intake is ± 0.5 SD above and below the mean; Low, net feed intake is < 0.5 SD above the mean.

a,b,c means in the same row differ, $P < 0.05$

Table 5. Energy requirements, methane and manure production from steers with high, medium and low net feed intake.

Traits	Net feed intake group[z]			SEM	Probability
	High	Medium	Low		
Number of steers	23	31	21		
Actual intake, kg DM/day	8.6a	8.2b	7.9c	0.08	0.0004
Net feed intake, kg/day	0.76a	-0.04b	-0.70c	0.07	0.0001
Animal requirements					
Maintenance energy[y], MJ/day	60.0a	56.1b	51.2c	1.2	0.0008
Maintenance intake[x], kg DM/day	5.45a	5.10b	4.65c	0.10	0.0008
LOI[w], kg DM/day	3.16	3.09	3.28	0.11	0.5716
Methane production					
Methane produced[w], % of GE	5.69	5.77	5.58	0.11	0.5716
Methane, MJ/day	9.62a	9.25a	8.62b	0.17	0.0069
Methane, g/day	174.4a	167.7a	156.6b	2.9	0.0069
Methane, g/kg gain/day	124.6a	115.6a	104.7b	3.7	0.0213
Methane, kg/550 kg gain	68.5a	63.6a	56.6b	2.0	0.0213
Methane credit[v], $ CAN/hd/yr	0.00	$0.77	$1.87		
Manure Production[u]					
Manure, kg/kg gain/day	2.03a	1.85b	1.73c	0.04	0.0024
Manure, kg/550 kg gain	1115a	1019b	953c	23.4	0.0024
N excreted, kg/550 kg gain	2.84a	2.60b	2.43c	0.06	0.0024
P excreted, kg/550 kg gain	6.13a	5.60b	5.24c	0.12	0.0024
K excreted, kg/550 kg gain	8.92a	8.15b	7.62c	0.18	0.0024

[z] Net feed intake groups are defined as follows: High, net feed intake is > 0.5 SD below the mean; Medium , net feed intake is ± 0.5 SD above and below the mean; Low, net feed intake is < 0.5 SD above the mean.

[y] The energy required for maintenance was calculated as heat production times metabolic mid-weight.

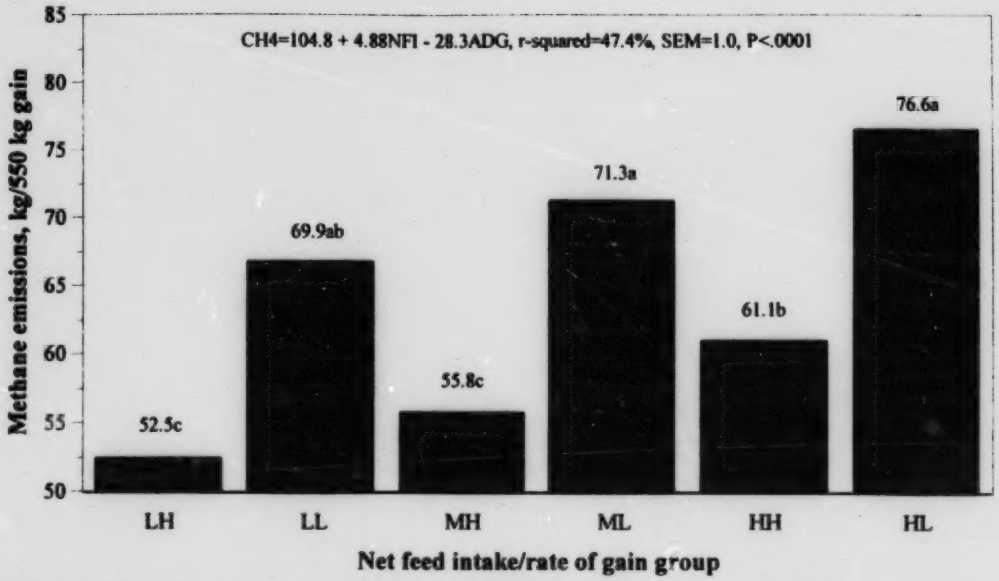
[x] The dry matter intake required for maintenance was calculated as maintenance energy divided by the ME of the diet (11 MJ/kg DM).

[w] Methane emissions are based on Blaxter and Clapperton (1965) equation: $\%CH_4$ loss (% of gross energy)=1.30 + 0.112DE + LOI(2.37-0.05DE, where DE is percent digestible energy (67.3%) and LOI is level of intake in kg above maintenance intake.

[v] One methane credit is worth $157.50 CAN/tonne of methane. This value is based on a carbon credit being worth from $5 US/tonne of CO_2 equivalents. Methane is 21 times more potent a greenhouse gas than carbon dioxide ($5 US/tonne x $1.5CAN/$US × 21).

[u] Manure production was calculated as follows: Yearly manure production=(((100-DM digestibility, %)/100) ×DM intake, kg/day) × 365. DM digestibility equals 67.3% as calculated from an in vivo digestibility trial. Nitrogen, phosphorous and potassium in manure were 2.55 kg/tonne, 5.5 kg/tonne and 8 kg/tonne, respectively.

a,b,c means in the same row differ, $P < 0.05$



LH=Low net feed intake, above average ADG; LL=Low net feed intake, below average ADG; MH=medium net feed intake, above average ADG; ML=medium net feed intake, below average ADG; HH=high net feed intake, above average ADG; HL=high net feed intake, below average ADG. Note: mean average daily gain was 1.46 kg/d; average mid-point body weight was 459 (SD=21) kg.

Figure 4. Relationship between net feed intake (NFI), average daily gain (ADG) and methane (CH_4) emissions from feeder cattle.

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Increasing Conjugated Linoleic Acid in Beef

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Key Points

- Components found in beef fat have positive health effects.
- Increasing levels of conjugated linoleic acid (CLA) in beef is beneficial to both consumer and producer.
- Research has shown that levels of CLA in meat can be significantly increased by altering diet.
- Future research should include studies to determine optimum CLA level in meat by manipulating cattle diets vs maximum effect on beef consumption.
- Future research should also include studies to determine age factors in the functionality of CLA supplementation.

Introduction

The profusion of research literature on the subject of conjugated linoleic acid (CLA) in recent years shows that there is much interest in CLA in terms of the functional properties of the compound and the benefits of those properties to human health. Since Pariza first discovered in 1984 that components isolated from fried ground beef had a positive effect on reducing tumours in mice (Pariza and Hargraves 1985), the CLA component of fat has been proven to have chemoprotective properties against cancer (Ip et al., 1991, 1994; Cornell et al., 1997; Sugano et al., 1997; Belury et al., 1997a; 1997b; Vanden Heuvel et al., 1999). However, CLA has recently been shown to also be effective against atherosclerosis (Kritchevsky 1997; Belury et al., 1997a, 1997b; Doyle 1998), obesity (Pariza et al., 1997; Delany et al., 1999; Park et al., 1999a, 1999b; Atkinson 1999), and diabetes (Houseknecht et al., 1998). Other recent research now suggests that CLA may also have an impact on enhancing the immune system (Cook et al.,

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1993; Hayek et al., 1999), combating allergies and asthma (Cook et al., 2000), and decreasing cholesterol (Carew et al., 1987; Nicolosi et al., 1997). Also, research has begun to narrow down possible modes of action for some of the functional properties. Thus, obesity may be decreased because leptin, a hormone that has been linked with obesity, is decreased by CLA (Rahman et al., 2001; Medina et al., 2000; Yamasaki et al., 2000). Diabetes is likely controlled because CLA normalizes the action of insulin in the body (Ryder et al., 2001). Even more exciting is the fact that research is also now beginning to show that not only does CLA protect against the above diseases, but in fact may have a reversing effect on some of them. Kritchevsky et al. (2000) found that just a 0.1% level of CLA in the diet inhibited atherosclerosis in rabbits. What is even more intriguing is the fact that a 1% dietary CLA level caused a regression of atherosclerosis. Furthermore, research has shown that adverse affects from increasing dietary CLA in humans are virtually non-existent (Benito et al., 2001; Scimeca 1998; Lowery et al., 1998; Kreider et al., 1998; Thom 1997).

Dietary CLA enhancement may also benefit the animals. Cook and co-workers (2000) discovered that they could combat a growth inhibition in chickens that occurs when they receive routine vaccinations. The growth inhibition is caused by the vaccine triggering the immune system, which in turn triggers an inflammatory response that releases proteins responsible for induction of muscle wasting. The chickens were fed a CLA mix and the growth inhibition was eliminated. The same response was noted in rats, mice and pigs. Cook's team has most recently been studying the effect of CLA on allergies and asthma. They have found, in studies soon to be published, that guinea pigs on a CLA enhanced diet have a dramatically reduced incidence of airway constriction (Raloff, 2001). They have determined that this reduction of the allergic reaction occurs as a result of CLA selectively depressing the activity of cyclooxygenase-2 (COX-2). Cyclooxygenase-2 is an enzyme that triggers a number of inflammatory responses. In fact, Cook's team have just applied for a patent on CLA as a COX-2 inhibitor.

As research progresses and continues to point to the beneficial properties of CLA, it becomes increasingly evident that enhancing levels of CLA in foods, in particular meats, is desirable. If beef producers can consistently provide a product with enhanced CLA to the consumer, both producers and consumers will benefit. Previous research (Mir et al., 2000a, 2000b; Dhiman et al., 2000, 1999) has shown that increasing levels of CLA over base levels in meat and milk is possible (Table 1). Continuing research will explore the effect of diet and sources of CLA in an attempt to optimize CLA levels in meat.

Are Other Meat Animals Ahead of Beef?

Despite all the positive results on CLA, research on beef cattle remains relatively limited. Just a handful of researchers have studied the effects of feeding CLA or CLA precursors to cattle (Geay et al., 2001; Flachowsky 2000; French et al., 2000; Pastushenko et al., 2000a, 2000b; Jahreis 2000) whereas the research on pigs (Bassaganya-Riera et al., 2001; Scheeder et al., 2000; Bee 2000a; 2000b; Glaser et al., 2000; Muller et al., 2000; O'Quinn et al., 2000; Dugan et al., 1999a; 1999b), dairy cattle and milk (Chouinard et al., 2001; Dhiman et al., 2000; Donovan et al., 2000; Griinari et al., 2000; Kraft et al., 2000; O'Shea et al., 2000; Piperova et al., 2000), chicken and eggs (Sell et al., 2001; Aydin et al., 2001; Du et al., 2001; 2000; Szymczyk et al., 2000; Simon et al., 2000; Ahn et al., 1999) lamb (Mir et al., 2000a) and goat's milk (Mir et al., 1999) is rather extensive (Table 2).

For the past several years we have been conducting studies to determine the effect of feeding sunflower oil on the fatty acid composition and CLA content of lamb and beef. In two of the studies we

fed cattle a finishing diet of 80% rolled barley and 20% barley silage. In the first trial, the animals were on one of two treatments (control and 6% oil) for 70 days. At the end of the trial muscle samples were taken to determine the fatty acid profile. CLA was increased by 17% in the animals fed the oil diet over the control diet (3.5 mg/g lipid for the oil group vs 3.0 mg/g lipid for the control group). In the second study, animals were fed one of three treatments (control, 3% oil and 6% oil). CLA in the steers fed the 3% oil diet was increased by 30% over the control diet (2.6 mg/g lipid for the 3% oil group, 2.0 mg/g lipid for the control group). Levels of CLA in the meat from the animals fed the 6% oil diet were increased by 75% over the control diet (3.5 mg/g lipid vs 2.0 mg/g lipid). In study three, three cattle breeds (Wagyu, Limousin × Wagyu, Limousin) were used and fed a backgrounding diet of 65% pea hay and 35% rolled barley. A finishing diet of 20% pea hay and 80% barley was fed after the backgrounding diet. Each of the breed groups was given one of two treatments (control and 6% oil). CLA levels in the Wagyu group were increased by 238% in the oil group (8.66 mg/g lipid for the oil group vs 2.56 mg/g lipid for the control group). The Limousin × Wagyu oil-fed animals had CLA levels 272% higher than the control group (5.92 mg/g lipid vs 1.59 mg/g lipid). In the Limousin group the results were even more remarkable. CLA levels increased by 354% in the oil group over the control group, with values of 12.98 mg/g lipid for the oil-fed steers and 2.86 mg/g lipid for the control steers (Mir et al., 2001) (Table 3).

Lambs were used in another trial (Ivan et al., 2001), where the finishing diet was 60% barley silage, 32% barley grain, 6% soybean meal and 2% mineral and vitamin supplement. Animals were on one of two diets (control and 6% oil). Samples of leg, rib and diaphragm muscles were collected at slaughter to determine CLA content. The level of CLA in rib muscle from oil fed lambs increased from 3.0 mg/g lipid (control group) to 5.2 mg/g lipid (33% increase), from 3.4 mg/g lipid to 4.8 mg/g lipid (37%) in the leg muscle and from 3.6 mg/g lipid to 5.6 mg/g lipid (56%) in the diaphragm muscle (Table 4).

In another study, conducted at the Lethbridge Research Centre with the support of Pioneer Hi-Bred International Ltd., the supplementation of sunflower seed at 14% of the diet dry matter improved performance of finishing steers relative to those not fed the sunflower seed. Conjugated linoleic acid levels were also increased in the brisket fat and diaphragm muscle, over the levels in animals on the control diet (22% and 18.5% respectively) (Gibb et al., 2001).

It is becoming increasingly clear that diet manipulation can have a profound impact on levels of CLA in the tissues of meat animals. It is also apparent that basal diet composition and days on feed also have an impact. To further demonstrate the effect of diet, muscle samples were collected from cattle on corn and barley diets. CLA levels were 125% higher in cattle fed corn diets than barley diets, even with no oil supplementation.

The Next Step: Getting Closer to Human Consumption

In 2001, we conducted a study using rats to determine if eating CLA- enhanced beef has an effect on body composition. Rats were placed on one of five diets. The first diet was a control, standard powdered diet (CON), using casein as a protein source, and soybean oil. The second diet was the same except for the use of sunflower oil as a source of linoleic acid (SUN), in place of the soybean oil. The third diet used 60% pure CLA in place of the oil (CLA). The fourth diet was a meat control (CON-meat)where the casein in the control diet was replaced with meat from beef cattle on a non-oil diet (CON-meat). The last diet (CLA-meat) was the same as the fourth diet, except that the meat used was from beef cattle on CLA-enhanced (oil) diet. Rats were fed the diets for six weeks and then terminated. Fat pads were collected for determination of weight, cell numbers, and CLA. Muscle and organ samples

were also collected for CLA determination. Although analysis is in progress, fat pad weights and cell numbers have been determined. Inguinal (groin) fat pad weights increased over the CON diet in the CON-meat and CLA-meat diets (18-23%), and slightly decreased in the SUN and CLA diets (2-5%). Retroperitoneal (gut) fat pad weights increased over the CON diet in every diet (11-37%) except the CLA diet, where they were decreased by 30%. These data indicate that the meat diets caused a noticeable increase, the sunflower oil caused a slight increase and the CLA diet caused a decrease in the weight of the fat pads.

Cell numbers from both the inguinal and retroperitoneal fat pads were increased over the CON diet in every diet (20-147%), but only very slightly in the CLA diet (4-5%). Taking into consideration the actual amount of CLA ingested by the rats, these numbers become clearer. CON diet rats ingested no CLA, SUN diet rats ingested negligible amounts, CLA rats ingested 396 mg/day, CON-meat diet rats ingested 5 mg/day and CLA-meat diet rats ingested 13 mg/day. So in fact, the CLA diet rats were getting 30 times the CLA that the CLA-meat diet rats were getting.

Cell numbers indicate the potential to store fat, in that each cell can increase in size, but the extent of fat storage capacity is limited by the number of cells in each fat pad. The greatest "potential" to gain weight was in the CON-meat diet where the cell numbers increased by 117-147% over the CON diet. The least potential was in the CLA diet where the increase over CON diet was negligible at 4-5%. When we look at the cell numbers for the CLA-meat group we see a noticeable increase over the CON diet, however, we also see that the cell numbers for the CLA-meat diet group were 40% less than those for the CON-meat diet. This indicates that the CLA-enhancement minimizes the fat storage of meat consumed. Since sunflower oil contains CLA precursors, and the monogastric animal is not capable of converting those precursors into CLA, the results for the SUN diet were as to be expected. There was a negligible decrease in fat pad weights and a noticeable increase in cell numbers over the control (Table 5).

Implications

From the above studies we can conclude that the levels of CLA in meat are easily manipulated by controlling the diet of the animal. The largest effect on increasing CLA levels in beef was in a hay/oil diet fed right from the backgrounding phase. We also see that higher levels of CLA produce more positive results in terms of controlling fat cell development and fat pad weights. What we suspect, is that CLA supplementation may be required at an early age to achieve optimum effects. The rats in our studies were fed CLA at the weanling stage and we noticed a positive effect in just increasing meat CLA levels from 5 mg/day to 13 mg/day. What research has not yet shown is the optimum levels of CLA to observe health benefits in humans. Because of the expense of manufacturing pure forms of CLA, it is difficult to increase dietary levels of CLA for human consumption to determine the optimum level. Our best option may be manipulating the diets of meat animals to increase levels of CLA in the meat. In turn, the CLA-enhanced meat may be marketable as a specialty or designer food.

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Table 1. Conjugated linoleic acid concentrations in uncooked beef.

Diet	Country	Concentration (mg/g fat)	Reference
Unknown	Canada	1.2 - 3.0	Ma et al. (1999)
Barley (800 g/kg diet)	Canada	1.7 - 1.8	Mir et al. (2000b)
Grass silage and conc.	UK	3.2 - 8.0	Enser et al. (1999)
Maize (820 g/kg diet)	USA	3.9 - 4.9	McGuire et al. (1998)
Unknown	USA	2.9 - 4.3	Chin et al. (1992)
Unknown	USA	1.7 - 5.5	Shantha et al. (1994)
Grain	USA	5.1	Shantha et al. (1997)
Concentrate	Japan	3.4	Tsuneishi et al. (1999)
Grass	USA	7.4	Shantha et al. (1997)
Grass (?)	Australia	2.3 - 12.5	Fogerty et al. (1988)
Grass	Ireland	3.7 - 10.8	French et al. (2000)
Unknown	Germany	1.2 - 12.0	Fritsche et al. (1998)

Moloney et al. (2001). Proc. Nutr. Soc. 60:221-229.

Table 2. Conjugated linoleic acid concentrations in various foodstuffs.

Foodstuff	# of samples	Total CLA (mg/g fat)	c-9,t-11 CLA (%)
Round beef	4	2.9 ± 0.09	79
Fresh ground round	4	3.8 ± 0.11	84
Fresh ground beef	4	4.3 ± 0.13	85
Veal	2	2.7 ± 0.24	84
Lamb	4	5.6 ± 0.29	92
Pork	2	0.6 ± 0.06	82
Chicken	2	0.9 ± 0.02	84
Fresh ground turkey	2	2.5 ± 0.04	76
Egg yolk	2	0.6 ± 0.05	82
Salmon	4	0.3 ± 0.05	not detectable
Lake trout	3	0.5 ± 0.05	not detectable
Sea scallops	2	0.3 ± 0.05	not detectable
Shrimp	2	0.6 ± 0.10	not detectable
Mussels	2	0.4 ± 0.04	not detectable
Homo milk	3	5.5 ± 0.30	92
Butter	4	4.7 ± 0.36	88
Plain yogurt	2	4.8 ± 0.26	84
Sharp cheddar	3	3.6 ± 0.18	93
Medium cheddar	4	4.1 ± 0.14	80
Cream cheese	3	3.8 ± 0.08	88
Brick	2	7.1 ± 0.08	91
Cheez Whiz™	4	5.0 ± 0.07	92
Safflower oil	2	0.7 ± 0.14	44 (41% t-10,c-12)
Sunflower oil	2	0.4 ± 0.02	38 (37% t-10,c-12)
Corn oil	2	0.2 ± 0.03	39 (37% t-10,c-12)
Olive oil	2	0.2 ± 0.01	47 (40% t-10,c-12)
Beef tallow	2	2.6 ± 0.01	84

Chin et al. (1992). J. Food Comp. Anal. 5:185-197.

Table 3. Effect of feeding sunflower oil on fatty acid and CLA composition in beef (mg/g lipid).

Fatty Acid	Finishing diet only					Backgrounding and finishing diets	
	70 days on feed		100 days on feed				
	Control	6% oil	Control	3% oil	6% oil	Control	6% oil
C16:0	331.9^{a}	316.1^{b}				320.5^{a}	276.4^{b}
C18:0	132.2^{b}	148.0^{a}	147.2^{ab}	144.2^{b}	155.0^{a}	84.6^{b}	113.7^{a}
C18:1	402.2	397.8	427.1	423.3	426.1	432.6	448.1
C18:2	13.8^{b}	20.3^{a}	15.7^{c}	21.2^{b}	24.7^{a}	13.8^{b}	24.2^{a}
C18:3	1.9	1.7	1.1	1.4	0.9	T	T
CLA	3.0^{b}	3.5^{a}	2.0^{c}	2.6^{b}	3.5^{a}	2.1^{b}	14.8^{a}

^{abc} means in the same row with different letters are different ($P < 0.05$).

Table 4. Effect of dietary sunflower seed oil supplement on the lipid and fatty acid concentrations in tissues of lambs.

Tissue	Tmt	Lipid	Fatty acid (mg/g lipid)									
		(%DM)	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:4	CLA
PCD muscle	Control	36.7	22.9	243	14.3	22.7	198	436	45.6	4.2	5.9	3.6
	Oil	42.1	25.3	222*	14.1	17.6*	227	412	61.6*	3.5*	7.4	5.6*
Leg muscle	Control	20.3	29.8	301	26.2	16.6	149	391	57.6	5.2	18.9	3.5
	Oil	18.8	28.5	254*	22.8	11.9*	155	405	92.4*	4.1	20.5	4.8*
Rib muscle	Control	32.9	30.7	289	20	20.3	185	399	39.1	4.1	6.7	3.9
	Oil	30.2	30.9	257*	17.6	14.9*	213	389	57.1*	3.2*	9.1	5.2*
Heart	Control	14.4	10.2	174	11.7	15.5	183	243	251	9.01	99	1.9
	Oil	14.8	12.6	159	9.9	10.6*	205*	213	268	5.10 *	113	2.1
Liver	Control	15.5	15.8	206	16.8	16.3	216	306	113	10	83.3	9
	Oil	15	10.0*	168*	12.8	10.2*	244	290	151*	6.7*	89.2	9.4
Kidney	Control	16.2	5.3	228	9.6	12.7	180	207	174	6.2	166	4
	Oil	16	3.7*	193*	10.1	8.1*	194*	189	218*	3.6*	170	4.5
Subcu. Fat	Control	96.9	43.9	291	31.6	24.9	159	406	28.8	3.8	0.9	5.4
	Oil	98.6	49.4	262*	29.3	20.3	177	408	36.5	3.8	0.8	7.2*

* significantly different from the control ($P < 0.05$).
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Table 5. Effect of diet on weight gain, fat pad weights and adipocyte numbers in rats.

Treatment	Weight gain (g) (P = 0.006)	Liver weight (g) (P = 0.07 trend)	Fat pads	Fat pad weight (g) (retroperi. $P < 0.0001$, trt effect) (inguinal P = 0.08, trt effect)	Cell # (10^6) (P = 0.0001)
Control	342 ± 9.9	17.9 ± 0.75	Inguinal	6.5 ± 0.57	0.97 ± 0.15
			Retroperitoneal	5.4 ± 0.47	1.03 ± 0.20
Control - meat	391 ± 9.9	19.8 ± 0.75	Inguinal	8 ± 0.57	2.47 ± 0.15
			Retroperitoneal	7.4 ± 0.47	2.2 ± 0.20
CLA - meat	351 ± 9.9	16.6 ± 0.75	Inguinal	7.7 ± 0.57	1.48 ± 0.15
			Retroperitoneal	6.6 ± 0.47	1.24 ± 0.20
Sunflower oil	348 ± 10.4	17.9 ± 0.79	Inguinal	6.2 ± 0.60	2.27 ± 0.16
			Retroperitoneal	6 ± 0.50	2.44 ± 0.21
CLA	343 ± 9.9	18.2 ± 0.75	Inguinal	6.3 ± 0.57	1.04 ± 0.15
			Retroperitoneal	3.7 ± 0.47	1.05 ± 0.20

Mir et al. (2001) (unpublished)

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The Importance of Value-Added Convenient, Consumer-Friendly Beef Products to the Future of the Beef Industry

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Key Points

- Beef lost market-share spending to poultry from 1980 to 1998, in part, because the poultry industry was so adept at developing and merchandising processed/prepared products that fit consumer needs for convenience.

- "Meal Solutions,"—generated via value-added processing—are necessary because preparation of a meal requires time and knowledge and are affordable because household incomes have climbed. Consumers prefer more conveniently prepared food products, take-home meals and home meal replacements and are willing to trade a portion of their bigger disposable incomes for food-preparer know-how and scarce discretionary household time.

- National Cattlemen's Beef Association (using beef checkoff dollars) and those in the beef processing industry have responded to market research by developing programs to make purchases of fresh beef easier and more nutritionally acceptable for consumers and by identifying, developing and/or marketing consumer-friendly, value-added and ready-to-eat beef products.

- Beef demand has increased in 9 of the last 11 quarters largely because of the industry's focus on delivering more convenient beef products, educating consumers about beef's nutrient bundle and producing safe and wholesome beef.

Introduction

Market-share spending for beef decreased in the USA, from 1980 to 1998, by 13.7 percentage points (from 53.9%, to 40.2%); almost all of the loss was attributable to an increase of 11.6 percentage points (from 16.1%, to 27.7%) in market-share spending for chicken (Smith, 2000a). Poultry meat, particularly chicken, became progressively more popular in the 1980s and 1990s because it was inexpensive, versatile, lean and nutritious and because the poultry industry was so adept at developing and merchandising processed/prepared products that fit consumer needs for convenience. Beef consumption has fallen among males and females of all ages over the past decade with only "older men (65+)" showing minimal (-7% in annual in-home beef eatings per capita, 2000 vs. 1991) losses, but beef maintains a dominant position, being consumed 40% more often than any other protein in the home (NPD/National Eating Trends, 2001).

Kay (2000) reported that market-share spending for beef stabilized and began to increase in the fall of 1999 and that the story of the year in 2000 was, without a doubt, beef's improved demand. Smith (2000b) quoted Bill Helming (Helming Consulting) as saying "It appears that the beef cattle industry is now putting into motion the necessary foundation of slowly taking back some of the

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market share lost to chicken over the past 25 years" and Wayne Purcell (Virginia Tech University) as saying "It's the most encouraging thing I've seen in 20 years; beef checkoff programs are working and so are the efforts of private firms which are putting out more convenient precooked products."

Beef (2001) said "Beef continues its upward demand growth; preliminary data from the first quarter 2001 indicates a 2% increase over the previous year's first quarter"; Texas Cattle Feeders Association (2001a) reported that beef demand increased in 9 of the past 11 quarters compared to the same quarters the previous year. Chuck Schroeder (CEO of National Cattlemen's Beef Association), in May 2001, said "Beef demand, which is a measure accounting for both per capita consumption and consumer spending for beef, has increased largely because of industry's focus on delivering more convenient beef products, educating consumers about beef's nutrient bundle and producing the safest beef in the world (National Cattlemen, 2001)". Henderson (2001) concurred, saying the success of the heat-and-eat beef entrées has helped the beef industry increase beef demand. And, Beef (2001) reported that demand has increased in part because, surveys indicate, beef's nutritional benefits continue to earn consumer recognition; a consumer attitudes study of 1,300 adults found 54% agreeing beef is an important part of a balanced diet and healthy lifestyle, compared to 51% in 1999.

Demand Drivers

Adolph (1999) said, "Consumers do not want your products and services...consumers only want their needs and wants met." At the World Meat Congress (Dublin, Ireland; May 1999), representatives of five countries described demand drivers in categories of economic concerns (cost; price/value), non-economic concerns (food safety; eating quality; diet/health/nutrition; convenience), ethical concerns (animal welfare) and ecological concerns (environment; food from genetically modified organisms) (Smith, 2000b). National Cattlemen's Beef Association (2000a) defined the "profitability drivers" in the beef industry as cost efficiencies and value enhancement and the "demand drivers" in the beef industry as food safety, palatability, health and nutrition, and consumer friendly products. National Cattlemen's Beef Association (2001) defined the "business climate drivers" in the beef industry as industry issues and image and beef production and the "demand drivers" as consumer confidence in beef (food safety, nutrition, positive eating experience), consumer friendly products (convenience, value) and beef exports. Fergal Quinn (SuperQuinn Supermarkets, Ireland) said at the International Livestock Congress in 2000, "So long as you see meat as a commodity, you will be driven by quantity—and so long as you do, the overall price trend will inevitably be downward; but, if you can get away from the straitjacket of regarding meat as a commodity, you tend to concentrate not on quantity but on how you can best satisfy the needs of the consumer" (Smith, 2000b).

Rocke (2001) said "Meal Solutions, generated via value-added processing, are necessary because people don't know how to cook any more. To prepare a meal requires time and knowledge, both of which have disappeared in the last two years. In-home meal preparation for breakfast and lunch disappeared first, and the move to Meal Solutions was very fast, because these meals occur at periods in the day when time pressures precluding meal preparation were greatest; Meal Solutions for breakfast and lunch have been dominated by processed products (including meat). And now, in 2001, meal preparation at dinner is moving that way—toward refrigerated, ready-to-eat, center-of-the-plate entrées—very fast. And, Meal Solutions broaden consumer exposure to new tastes and new meats because value-added processing removes the danger of experimentation and the potential for messing-up an expensive piece of meat."

Berry (2001) said, "A glance at the past century's food-development milestones suggests an industry driven by consumer demands for convenience, particularly interest in receiving assistance in the kitchen. Convenience, however, is a dynamic term that seems to change with each generation;

today's consumers want more than consumers did in 1930, when they responded so favorably to Wonder® Bread, the first, packaged, sliced loaf of bread. Fewer consumers know how to cook, or are willing to take the time to learn. Consumers also are well traveled, and thus desire food with a global flair. Because home is still the preferred place to eat dinner, product developers are busy designing a new generation of speed-scratch meal components to make it easier to prepare a dinner with a hint of home-cooked flair." Nearly 40% of the consumer's food dollar is spent in restaurants and other eating establishments—the ultimate in food preparation convenience and consistent dining quality (ERS-USDA, 1999). Other evidence indicates that the quantity of food consumed away-from-home has not risen as rapidly as expenditures on food away-from-home (Barkema et al., 2001). Carlson et al., (1998) reported that nearly three-fourths of the quantity of food consumed is believed to be purchased in grocery stores.

Market Research On Food Selection Concerns

According to the Market Link Shopper Survey (Food Processing, 1999) the rank (10-point scale) of "Influences on Purchases of Food Products in the Supermarket" were: (1) taste, 9.61; (2) what my family wants/prefers, 8.64; (3) price, 8.17; (4) nutrition, 8.06; (5) product convenience, 7.29; (6) package size/portion I need, 7.01; (7) information on package, 6.92; (8) coupon, 6.52; (9) brand name, 6.29; and (10) easy-to-use package, 6.23. Smith and Morgan (1999) reported "Six questions asked by consumers as they make decisions about purchasing meat," according to Steve Harper (HEB Supermarkets), are: (1) Will it hurt me? (food safety): (2) Is it good for me? (diet/nutrition/health); (3) Can I afford it? (value); (4) Do I know how to fix it? (cooking/preparation); (5) Do I have time to fix it? (convenience); and (6) Do I like it? (preference). According to Food Marketing Institute (1988, 1992, 1996, 1998, 2000a) the percentages of supermarket shoppers that considered specific factors "very important" in food selection are presented in Table 1. This question was not asked in the 2001 edition of "Trends in the United States; Consumer Attitudes & The Supermarkets" but "taste" ranked first in all 13 years of these "Trends" analyses while "nutrition," "product safety" and "price" were always 2nd, 3rd or 4th but not always in the order they were ranked in 2000. In those "Trends" analyses, "price" tended to rise or fall depending upon the economic state in the USA at the time (year) while "nutrition" and "product safety" changed in relative ranking depending upon media coverage of those issues at or near the time of the interviews.

Sloan (2000) said, with 98 million adults afflicted with high cholesterol, 70 million at risk from blood plaque/triglycerides, 50 million with high blood pressure, and 114 million overweight, consumers are looking for wellness and health maintenance when they shop for food. In 1998, for example, 23.68 million households tried to manage heart-related conditions, while 33.69 million were trying to treat them through grocery purchases. In 1999, 55 million (57%) primary grocery shoppers were highly/moderately involved in maintaining their own health, and 52% said health concerns greatly affected their food purchases (Sloan, 2000). Healthful fast-food options exist (Waldsmith, 1999). Eating at home isn't always an option, which is why fast food is so popular; while it is not easy to integrate fast food with healthful eating, some choices at fast-food restaurants are better than others. Waldsmith (1999) quotes a registered dietitian at Henry Ford Heart and Vascular Institute (Detroit, MI) as saying "The best choice is the plain hamburger; it's lower in fat. The amount of cheese on the cheeseburger is small but it's loaded with fat." Harry Balzer (The NPD Group) said (Food Systems Insider, 2001b) "The following trends have permeated eating habits in recent years—1985, microwave ovens; 1990, low-fat foods; 1995, Home Meal Replacements; 2000, weight loss. Ten years ago, many consumers turned to foods that were low in fat but people found that many of the low-fat foods didn't taste good. And, they also found that low-fat foods alone would not accomplish the trick; many other factors, including exercise, are vital to promoting a healthy lifestyle. While fat content is less of a concern today, people still want to lose fat. In fact, weight-loss has become the No. 1 dietary trend."

Consumers continue to be time-pressed and are looking for solutions to the time spent in meal preparation; the food industry continues to generate products that meet consumer's desires to save time in meal preparation and to eat on the run (Food Marketing Institute, 2001). "Americans now want instant everything, whether it's cooking or eating, cleaning house or pumping gas, people want to make life in the fast lane even faster; whatever the cause, marketers have flooded the marketplace with an explosion of products designed to speed things up" (Washington Post, 2001). Barkema et al., (2001) reported that with consumers seeking more convenient dining options, traditional food retailers are locked in a competitive battle with restaurants and other food service establishments, boosting their offerings of prepared or ready-to-eat foods. Such selections were sparse a decade or two ago, but by the late 1990s more than 80% of the nation's supermarkets sold prepared foods, including sandwiches, pizza or pasta dishes (Kaufman, 2000). Some industry observers indicate that an especially promising development area for new products is the "Convenient Meal Solutions" category that provides consumers time-saving approaches to meal preparation; as a result, the number of new food products each year soared to nearly 17,000 in 1995 before tapering off more recently (Barkema et al., 2001).

Market Research On Shopper's Eating Habits

When asked "How often do you do certain things relative to eating habits?", supermarket shoppers responded in the manner displayed in Table 2 (Food Marketing Institute, 1997, 1999, 2001). Among women supermarket shoppers working more than 20 h per week, 40% use prepared foods that you heat- and-eat as compared to 28% of women working 0 to 19 h per week who use heat-and-eat foods (Food Marketing Institute, 2001). While consumers may be eating meals out less often, dining in may not be a home-cooked meal; eating home-cooked meals at home has been declining since 1998 (Food Marketing Institute, 2001). According to Berry (2001), Jon Hauptman (Willard Bishop Consulting Ltd.) explained at the 2001 Specialty Food Distributors and Manufacturers Association Annual Conference that: (a) Consumers are looking for quick, creative ways to cook dinner for their families at home; (b) "Homecooked" no longer means "from scratch"; research indicates that 60% of families eat at home five to seven times per week, with 44% of weekday meals prepared in 30 minutes or less; (c) Interestingly, 60% of the home-prepared meals do not involve a side dish and 69% of meal preparers are looking for ways out of the "recipe rut."

Market Research On Sources Of Takeout Foods

Working women (often with children) are under special time-pressures; shoppers cope with this increased pressure by shortening meal preparation time through the use of convenience foods and takeout foods and eating meals out (Food Marketing Institute, 2001). According to NPD Group, Inc. (2000), over 44% of weekday dinners are prepared in less than half an hour. Based on responses to the question "When meals are eaten at home but not prepared at home, are they purchased most often from a fast-food restaurant, a restaurant, a supermarket, convenience store, gourmet or specialty store or some other place?" (Food Marketing Institute, 1988, 1996, 2001) sources of takeout foods (Table 3) were: (a) Fast-food restaurant, 32% (down by 16 percentage points in the last 5 years); (b) Restaurant, 23% (down by 15 percentage points since 1988); and (c) Supermarket, 20% (almost double the 11% in 1988). When asked, "How often do you use prepared foods for main dishes that you just heat and eat?", 12% of supermarket shoppers said 3 or more times a week, 26% said 1 or 2 times a week, and 26% said 1 to 3 times per month (Food Marketing Institute, 2001). When asked, "How often do you use short-cuts such as bagged salads, pre-cut and cleaned vegetables or marinated meats?", 17% of supermarket shoppers said 3 or more times a week, 24% said 1 or 2 times a week, and 22% said 1 to 3 times per month (Food Marketing Institute, 2001).

The majority of U.S. women (51%) between the ages of 25 and 39 still make almost every evening meal at home from scratch, according to a survey conducted by the Canned Food Alliance (1999) and reported in Prepared Foods (1999b). That does not agree with Food Marketing Institute (2001) which, in response to the query "When you prepare main meals at home, how often do you make meals from scratch, using fresh ingredients?", reported that supermarket shoppers said: (a) 3 or more times a week, 48%; (b) 1 or 2 times a week, 22%; (c) 1 to 3 times per month, 14%; (d) Less than once a month, 8%; and (e) Never, 5%. "Despite all that we have heard about Americans cooking less and eating out more frequently" says cookbook author and Bon Appetit columnist Melanie Barnard, "we still place a high premium on the importance of preparing family meals and eating together every night" (Prepared Foods, 1999b). What shoppers mean by home cooking is not necessarily "made from scratch" meals. "Home-cooking" may be a meal cooked at home but it may be made using a variety of convenient short-cuts; these short-cuts range from meals that you just heat-and-eat, and marinated meats or bagged salads, to boxed or canned meals to which you add some other ingredients (Food Marketing Institute, 2001). Prepared Foods (1999b) says "On average, all respondents eat dinner at home nearly six nights per week, 91% have children living at home and 71% work outside the home at least part time." The majority described themselves as "Suzy Homemakers" and 41% categorized themselves as "Prepared Foods Paulas," relying on prepared foods and leftovers to accommodate their family's busy schedules. More than 4 out of 5 (84%) also use takeout food as a fallback on really busy nights, with pizza being the overwhelming takeout food of choice (Prepared Foods, 1999b).

According to "Trends in the United States—Consumer Attitudes & The Supermarket 2001" (Food Marketing Institute, 2001), the number of times per week consumers eat the main meal away from home is: (a) Once, 34%; (b) Twice, 18%; (c) Three or more times per week, 15%; (d) Never, 13%; and (e) Less than once, 17%. When asked, "How often do you eat meals at home that are not prepared at home, like takeout or home delivery" supermarket shoppers (Food Marketing Institute, 2001) responded: (a) 3 or more times a week, 6%; (b) 1 or 2 times a week, 25%; (c) 1 to 3 times per month, 33%; (d) Less than once a month, 20%; and (e) Never, 15%. Barkema et al., (2001) reported that: (1) demographic factors account for much of consumers' new penchant for dining convenience; (2) a telling indicator is the increased participation of women in the American work force, which climbed from less than half in 1970 to nearly two-thirds more recently; and (3) as a result, household incomes have climbed and household schedules have tightened; thus, consumers prefer more conveniently prepared food products, trading a portion of their bigger disposable incomes for scarce discretionary household time. Kinsey et al., (1996) reported that rising consumer incomes and wage rates that exceed marginal productivity of household production of food, plus a decrease in the real cost of food by one-third since 1960, lead to a greater demand for value-added services in the food sector.

Market Research On Consumer Eating Trends

Balzer (1999), using results of the 1998 National Eating Trends report and the CREST study, found that: (a) diet/health/nutrition concerns are declining from "high-water marks" in 1991-1993; consumers are less concerned in 1998 than they were in 1988 about salt, fat, preservatives, cholesterol, sugar and caffeine; (b) the major result of the diet/health/nutrition craze of the last two decades is that our animals got skinnier (because of emphasis on leanness in cattle, swine and sheep) while our people got heavier and fatter; (c) health trends have not disappeared, they've moved to diet/health/nutrition solutions via vitamins and nutraceuticals (Knox Gelatin is now sold as Knox Nutra Joint); (d) we are frying foods less often but eating more fried foods; (e) side dishes for main meals are disappearing (2.9 per meal in 1987; 2.5 per meal in 1998); (f) vegetarianism is not increasing much; 3% in 1987 vs. 4% in 1998, do not eat meat; (g) of meals prepared in the home vs. outside the home, the amount is 50:50 but the cost is 30:70; (h) in the 1970s, 80% or more of women

prepared at least one meal per day in the home, in the 1980s it averaged 74% and in 1998 it is 68%; and (i) of 1,095 opportunities to eat a meal in the year of 1998, 682 (62%) were prepared and consumed in the home; 44.7% of those, in 1985, were main meals prepared from scratch while, in 1998, 38.4% were main meals prepared from scratch (Balzer, 1999). NPD/National Eating Trends (2001) reported that: (a) the number of U.S. women over 18 increased by over 10 million in the past decade; there are presently 106.7 million of them; (b) 60% of all women work outside the home; (c) women are still responsible (71% to 79% of the time) for the planning, shopping, preparing and cleaning-up of dinner meals in America; (d) 43% of homemakers say the most important things about foods are that they look good, smell good and taste good; and (e) 14% of homemakers completely/mostly agree with the statement "Convenience is most important in the foods I eat."

Market Research On Food Trends

Balzer (1999) followed food diaries for a housewife (in 1955), that same woman later in life (in 1997), and that of her daughter, now a housewife (in 1997), for a story in the Washington Post in 1999. The housewife in 1955 prepared 21 of 21 meals in a week in the home, took 29 minutes to prepare the average meal and served coffee as the primary beverage at those meals. Forty-two years later that housewife prepared 11 of 21 meals, took 9 minutes to do it and served primarily soft drinks. But, her daughter prepared 18 of 21 meals in a week in the home, took 20 minutes to prepare the average meal and served coffee as the primary beverage at those meals. The point is that people, individually, change as they proceed to a new life stage but people of the same life stage don't differ much in what they do compared to what their parents did at that stage in their life (Balzer, 1999).

The ten most popular, supper main dishes in 1990 (Balzer, 1999) were: (1) spaghetti, (2) steak, (3) soup, (4) fresh baked chicken, (5) pizza, (6) hamburger, (7) pork chops, (8) fresh seafood, (9) salad, and (10) fresh fried chicken. In 1998, the list reads; (1) pizza, (2) spaghetti, (3) soup, (4) fresh baked chicken, (5) steak, (6) hamburger, (7) pork chops, (8) salad, (9) fresh seafood, and (10) frozen entrée (the only new entry). Balzer (1999) interprets this to mean that: (a) the single most important thing about our diet is familiarity; (b) emphasis in diet/health/nutrition is changing (less concern about fat, calories, cholesterol; more concern about taste); Americans are attempting to improve their health by use of vitamins and nutraceuticals; and (c) when changes in the U.S. diet occur they are due to growing population segments in new lifestages (e.g., empty-nesters), price, time-savings or fads/fun ("new" experiences). Balzer (1999) says it all boils down to the consumer saying: (1) Save me money, (2) save me time, and/or (3) make the "old" (familiar products) "new."

The "Top 15 Food Trends For 1999-2000," called "15 Ballpark Bets" by Prepared Foods (1999a) are: (1) The Natural (organic and all-natural foods); (2) Enroute To Ensemble (nutritionally enhanced/functional foods); (3) Packaging For Seniors (bottles and bags that actually open); (4) Voluptuous Sells (focus on taste, irrespective of fat content); (5) Rip HMRs (no one wants a HMR; they want convenient products); (6) Function Conjunction (functional foods, not beverages); (7) Three Little Pigs (bacon is in; in chips, dips, salad dressings, side dishes); (8) Betty Crocker Branches Out (into casseroles, pastas and cup dishes); (9) Hispanic Happenings (more products with spicier ethnic flare); (10) Vanilla: Flavor of the Year (wide range of upscale, elegant, sought-after flavor); (11) Brazilian: Cuisine of the Year (beef products with chimmichurri sauce); (12) www.watchout.com (Internet as a legitimate contender for consumer food dollars); (13) One Big Mart (Kmart, Wal-Mart will enter traditional supermarket business); (14) The Force is With Tie-Ins (a bonanza of Star Wars™ products and tie-ins); and (15) Y2K XS (excess of products claiming "product of the millennium").

Balzer (2000) reported that: (a) the number of meals prepared and eaten at home (N=917) is at an all-time low; meals at restaurants (N=139) are at an all-time high; (b) of the 14% increase in

restaurant meals in the decade of the '90s, fast-food restaurants captured 80% of that growth; (c) heat-and-eat meals are becoming increasingly popular; in 1999, 34% of all home-cooked meals included at least one item where preparation was just "warmed" or "heated"; (d) consumers are more concerned with their time and money than with maintaining a balanced diet; and (e) after years of attempting to eat our way to better health, with the only tangible result being a heavier American, we are looking for the easiest and cheapest way of feeding ourselves. Wall Street Journal (2001) recently reported that "Just in time for a shrinking economy, culinary downsizing has arrived. After years glued to the Food Network® and weekends spent buying fancy cooking equipment, wannabe chefs around the country are facing a moment of truth. They're realizing they're not just too lazy to cook; they don't have the time or even the inclination to do so. The result: a small but growing number of Americans aren't only downsizing their kitchens, sometimes they're tearing them out altogether."

Kay (2001) citing the June 2001 Issue of Food Technology identified, as the top ten food trends of the future: (1) Do-it-For-Me Foods—Pre-made meals and take-out; (2) Super Savory and Sophisticated—Baby Boomers with high disposable income want these foods; (3) Balance—There is now less obsessing about low-fat and high-fiber foods; (4) Form Follows Function—Bits, bites and appetizer foods; (5) A New Kind of Home Spun—One-dish meals that can go from oven to table to dishwasher; (6) Kid-Influenced—One in four kids is overweight; (7) Light and Lively—Foods that are fresher and have a more natural image; (8) Crossover Meal Patterns—Soups and sandwiches because of more snacking and varied mealtimes; (9) Do-it-Yourself-Health—Foods that are fortified, functional and performance-enhancing; and (10) Clean, Pure, Natural, Safe—One-third of grocery shoppers buy organic foods.

Today's time-conscious consumer has turned the deli into a destination for take-home meals and convenience foods; whether it's called "Home Meal Replacements" or "Meal Solutions," on average, supermarket-deli prepared meals are purchased for dinner 1.1 times per week—1.3 times by men and 1.0 times by women (Food Systems Insider, 2001c).

Market Research Related Specifically To Processed/Prepared Beef Products

Properties influencing consumer demand for processed/prepared beef products (Smith et al., 2000) include: (a) novelty (new and different, changed in form, modernized, with added value); (b) quality (taste, tenderness, physical attractiveness, storage stability); (c) simplicity (quick, fast, time-saving, uncomplicated); (d) convenience (easy to prepare, easy to serve, easy to clean-up after); (e) safety (bacteriologically safe, chemically safe); and (f) consistency (sameness in appearance from purchase to purchase, sameness in performance from preparation to preparation, sameness in palatability from eating experience to eating experience). Smith (2000c) said the need for such properties in processed/prepared beef products is justified because: (a) beef's primary competitor—chicken—has changed the form in which it is marketed, from 7% (in 1975) to 43% (in 1998) further-processed product; (b) 70% of women are in the U.S. work force and 28% to 58% of women and men say they have "lack of time" or "too busy schedules" to cook, because of their lifestyle; (c) two-thirds of all dinner decisions are made the same day; of those, 73% of dinner preparers don't know at 4:30 PM what they're going to have for dinner; (d) simplicity and convenience are the most important factors driving meal preparation decisions; (e) 41% of consumers under the age of 25, and 50% of those aged 26 to 35, say their lack of cooking knowledge keeps them from buying certain cuts of beef; (f) to satisfy home and food-service preparers, beef must be made easier to purchase, prepare, place on a menu, fit into consumers' busy lifestyles and fit into changing food-service environments; and (g) during the week, 40% of households spend less than 30 minutes on meal preparation while 78% of households spend less than 45 minutes on meal preparation. Smith (2000b) quoted Wayne Purcell (Virginia Tech University) as saying "Beef prices to consumers need to go up, and go up on the strength of value-added activity, new product offerings, and modernized product lines. Boosting

prices by offering consistent, delicious and easy-to-prepare beef products and by cutting cattle/beef production costs are complementary and both are needed."

Berry (2001) said consumers spent more for beef in 2000 than at any other time in history ($52 billion, 3.3% more than in 1999) much of it at steakhouses; because consumers are enjoying the taste of beef when they dine out, they now want to experience it at home. The beef industry is working with food manufacturers to develop different entrées that make it easier for consumers to prepare beef for dinner. In the past year, a variety of heat-and-eat products have debuted in the meat department—microwaveable pot roast, barbecue-beef tubs, premarinated chuck steak, seasoned meatballs and cooked ground beef crumbles. All these heat-and-serve beef products are helping grow the beef category; since 1998, convenient beef-product retail sales grew from $43.22 million to $84.27 million, a 95% increase. Existing items accounted for a 7% increase (or $3.8 million) in retail sales in 2000, as compared to the previous year; the rest of the category growth came from new products, which generated $27.8 million (Berry, 2001).

Industry Reaction To Market Research

What do consumers value? Thomas (2000) concluded that the two most important demand drivers for beef are: (a) convenience—consumers must believe that beef is easy to use; and (b) nutrition—consumers must feel that beef is easy to choose. Food Marketing Institute (2001) asked the question, "Can healthy food and convenience co-exist?" They say: the need for convenience often translates into making food choices that are not as good nutritionally; in "Shopping for Health 2000, Self-Care Needs and Whole Health Solutions" (Food Marketing Institute, 2000b), the majority of single-parent shoppers agreed that most healthy foods are not convenient to make. In "Trends in the United States—Consumer Attitudes & The Supermarket 2001" (Food Marketing Institute, 2001) the youngest shoppers reported an interest in nutrition while at the same time they are the age group most likely to turn to fast-food takeout and the least likely to undertake home-cooking; this is an apparent contradiction in attitudes and behavior. But, there is evidence that healthy food and convenience can co-exist. Food Review (2001), citing a doubling of the sales of produce from 1987 to 1997, says this rise in consumption occurred because of increased interest in nutrition (as promoted through programs like the Food Guide Pyramid) plus the produce industry's effective response to consumers' need for convenience (items such as packaged salads and fresh-cut produce meet consumers' demand for quick-and-easy, healthy food).

Beef was losing the "value proposition" to chicken for twenty years. However, in June 2000, Tyson Foods reported low third-quarter earnings "because of weak chicken prices," and in July 2000, David Nelson (First Boston Bank) said "The revival of the red meat industry and demand growth for red meat (it's stronger than we've seen in 20 years for beef) have occurred because of better marketing and because the beef industry has helped people know how to prepare cuts in the right way." Thomas (2000) described the National Cattlemen's Beef Association's "Beef Must Be EZ To Use; Beef Must Be EZ To Choose" efforts, emphasizing the following: (a) today's meat case is a "sea of red," with confusing names, no instructional help for shoppers, selling a limited range of cuts and with random product placement; (b) tomorrow's meat case must be set the way consumers think, to speed their purchase decisions and increase their confidence in buying a broader range of cuts; that will make it easier to stock and will increase overall beef sales; (c) the target audience is women, 25 to 54 years of age, with children at home (there are 59 million of these in the population of the USA); and (d) what we want consumers to believe about beef is that it is nutrient dense and an important component of healthy diets, that beef and poultry are nutritional equals, that beef is important to long-term health and that beef is safe and wholesome. Thomas (2000) identified "convenience" as a demand driver for today's beef consumer and qualified that decision by describing the target consumers as follows: (a) primarily female, 25 to 54 years old, in a two-income household, (b) strong

need to satisfy and take care of their family, (c) time spent with the family is a priority, (d) may know how to cook, but lack the desire to spend time cooking, and (e) don't want to waste valuable time or energy on meat decisions.

To make beef "easy to use," it has been advantageous for supermarkets to change the face of presentation of fresh beef at supermarkets. Berven and Tully (2000) described the "Beef Made Easy" program of Kroger Supermarkets as: (a) setting the beef case by the way the consumer thinks, (b) cuts are given color-coded labels, by recommended method of cookery, (c) cooking instructions are provided on each package, and (d) iron-man placards, rail strips and on-pack labels emphasize cookery method (e.g., "Pot Roast," "Oven Roast," "Steaks For Braising," etc.). HEB Supermarkets has taken cookery method on-pack instructions one step further (Harper, 2000) by conducting in-house research studies that allowed them to develop cooking instructions unique to anatomical origin, size (weight, thickness) and processing (e.g., cubing) or preparation (e.g., marination) procedures. According to Wood (2000b), "Initiated in 1999, 'Beef Made Easy' reorganizes the beef section of the meat case by cooking method, which is more in line with how consumers shop. It makes beef easier to prepare by providing color-coded cooking instructions on packages of beef, as well as incorporating easy to find convenience sections for heat-and-serve products. At least 23 of the country's top 34 supermarket chains tested or implemented the 'Beef Made Easy' program in its inaugural year. Results of 'Beef Made Easy' tests, conducted last year with Eastern and Midwestern USA supermarkets, showed a first-month increase in tonnage-moved of 7.9% and 4.1%, respectively". Food Systems Insider (2001a) reported that the NCBA's "Beef Made Easy" program, reorganizing the meat case by cooking method, has been implemented in 20 grocery store chains and 6,000 stores nationwide.

The year 2000 saw a 29% increase in refrigerated entrees; many beef manufacturers see this category taking off and major players such as Lloyd's, Louis Rich, Hormel and Oscar Mayer are starting to enter into it by developing and marketing their own new beef products (Smith et al., 2000). In the last year, we have seen that retailers who have created convenience sections in the fresh meat case for further-prepared, value-added items are realizing greater sales returns. With increased sales of these products made from underutilized cuts from the chuck and round, the value of the overall carcass is ultimately enhanced (Wood, 2000b). According to Olson (2001b) "Branded beef products, many of them convenience-oriented, are flying out of the grocer's meat case. In the last 3 years, 300 new beef products have come to market; awareness of heat-and-serve beef products among mothers of ages 25-54 rose to 62% last year, a 20% increase from the year before. Heat-and-serve beef products, many made from the chuck and round have increased the value of the round by 10% (last year) and the chuck was up 17% from last year, according to Cattle•Fax data."

Once the market research had been carefully gathered and assessed, and it was determined that new forms of a product were needed to meet consumer demands, a question arose regarding whose responsibility it was to assure that research/development occurs, that appropriate products were identified, and that these products were manufactured and strategically marketed? One school of thought is that beef checkoff dollars should be directed toward: (a) fundamental meat science research, (b) technological research to facilitate manufacture of products, (c) identification of specific products and research/development to describe manufacture of such products, and (d) marketing and merchandising of specific products. Another school of thought is that beef checkoff dollars can be directed to (a), (b) and (d) above, but that—because meat companies desire exclusivity with regard to development and manufacture of new beef products—the private sector must perform (c) above. National Cattlemen's Beef Association, using checkoff funds administered by the Cattlemen's Beef Research and Promotion Board, has done some of all four of these things.

To make beef "easy to use," it has been advantageous for the beef industry to conduct research to characterize properties of certain muscles that could be used to manufacture value-added, consumer-friendly, further-processed and ready-to-eat beef items. National Cattlemen's Beef Association (2000b) has conducted research at the University of Nebraska and the University of Florida and developed a manual ("Muscle Profiling") that is a compilation of chemical and physical traits of 39 muscles from the chuck and round. The manual includes recommendations from several industry task force input sessions and is designed to aid packers, processors, purveyors, researchers/developers, retailers, food-service operators and others as they supply, process and develop new beef products from the chuck and round. Included in the manual, for 27 muscles from the chuck and 12 muscles from the round, are: (a) physical traits (length, width, minimum/maximum depths, weight, percentage), (b) chemical characteristics (pH, expressible moisture percent, emulsification capacity, heme iron content, collagen content), (c) color measurements (L*, a*, b*), and (d) shear force values (for muscles cooked by use of both moist heat cookery and dry heat cookery). Data were collected from carcasses in three Quality Grades, four Yield Grades and three carcass weight ranges. The rationale for conducting the study was that prior economic research, conducted by Cattle•FAX, revealed that there had been a 20-30% decline in the value of the beef round and beef chuck in the five years prior to initiation of this study (National Cattlemen's Beef Association, 2000b). The stated purpose for conducting the study was to characterize individual muscles from the chuck and round with the ultimate goal that such knowledge would be communicated to all sectors of the beef industry and with the intention that the optimal uses of each muscle can be found, thereby returning the optimal value (National Cattlemen's Beef Association, 2000b).

At Cattlemen's College, NCBA convention, Calkins and Johnson (2001) described the "Muscle Profiling Study" as follows: (a) the ultimate goal of the study is to add value to the chuck and round; (b) information about each muscle from the chuck and round will facilitate capture of the greatest possible value for every muscle; and (c) finding alternative uses for chuck/round muscles allows targeted product enhancement, increases demand for underutilized cuts and take pressure off middle-meats (loins and ribs). Lusk and Meade (2001) described retail applications of the "Muscle Profiling Study" as follows: (a) retailers and food-service operators lack the resources to conduct such studies, yet need such information for new product development opportunities, and (b) retailers and food-service operators can use data from the study to select raw materials to match attribute needs in fresh steaks and roasts, seasoned/enhanced/marinated, or fully cooked ready-to-eat beef products.

To make beef "easy to use," it has been advantageous for the beef industry to support research by a consulting firm to develop specific ready-to-eat beef items. Henderson (2001) quotes Mark Thomas (NCBA) as saying "Heat-and-serve beef entrées are gaining popularity because they start with a significant product offering (such as a pot roast) and add the convenience factor (doing things for people that) most people won't do for themselves." Because "simplicity" and "convenience" are the most important factors driving meal preparation decisions, NCBA, through activities of its R&D Ranch affiliate, has developed (Smith, 2000b) seven new convenient beef products: (1) Boneless Beef Filet (from the clod), (2) Beef Ham (from the chuck roll), (3) Rotiss A Roast (from the top blade), (4) Steak Tortilla (from the sirloin tip), (5), Today's Roast (from the knuckle center), (6) Cheeseburger Fries (a new form of ground beef), and (7) Ground Beef Crumbles (precooked ground beef). The extent to which commercial concerns will seek to capitalize on these ideas has not been determined but the option to do so exists. The beef industry's focus on convenience products is starting to pay big dividends; measured against total retail beef sales, the new heat-and-serve beef entrées remain a relatively small category in retail outlets (Henderson, 2001). But, the heat-and-serve category generated sales of $84 million last year, in only its third year as a separate category; retailers expect rapid growth over the new few years. There has been a significant increase in the number of major national beef marketing companies that now have products for the

heat-and-serve category including IBP, Farmland Foods, Hormel, Burnett & Sons, Harris Ranch, Pillsbury and Kraft (Henderson, 2001).

There are consumer-friendly, value-added and ready-to-eat beef products that have been developed by packers and processors. Brad Caudill (Harris Ranch Beef Alliance) said his company developed a Beef Tri-Tip Roast (fully cooked, 2 lb each, feeds 4 people) from the bottom sirloin in 1992, because, "We had to change, or die. To meet consumer needs, sure; but also to add value for the packer and add value for consumers (convenience, home meal replacement, grab-and-go, speed of preparation)" (Smith, 2000b). Since introduction of their first successful value-added product, the Harris Ranch Beef Alliance has added Beef Pot Roast in 1995, Beef Stew in 1997, Beef Short Ribs and Beef Swiss Steak in 1998...and, four more products are on the way for 2000-2001 (Smith, 2000b). Smith (2000b) reported that: (a) in 1999, Certified Angus Beef (CAB) was sold in 459 restaurants and 495 supermarkets; (b) among CAB Heat N' Serve products are "Prime Rib Slice," "Top Sirloin Steak," "Beef In Barbecue Sauce," "Beef Tri Tip Roast," "Beef Pot Roast" and "Beef Meat Loaf" (all microwavable in 2 to 10 minutes); (c) among CAB Ready To Prepare Entrees are "Frankfurters," "Bubba Burgers," "Knockwurst" and "Thick N' Juicy Beef Patties;" and (d) among CAB Deli Selections are "Roast Beef," "Bologna," "Corned Beef," "Pastrami" and "Beef Summer Sausage."

Olson (2001a) reported that the beef industry has ground up some of its profit potential for years but a new check-off funded project shows retailers and foodservice operators how to cut the carcass differently and make steaks instead of burgers. "Beef Value Cuts: New Cuts For The Consumer" shows end-users how to recapture some of the lost value in the chuck and round that was identified in the "Muscle Profiling Project." The result is 16 new steaks, 15 of which meet USDA's "lean" or "extra lean" requirements; traditionally, these cuts have had more fat in them, but the fabrication technique to extract them removes most of the fat (Olson, 2001a). Carl Blackwell (Executive Director of New Product Development, NCBA) says "The Beef Value Cuts program helps manufacturers improve the quality and consistency of their raw material and provides more solutions for consumers—whether they be case-ready products, value-added fresh beef, fully cooked items or frozen concepts" (Olson, 2001a).

The beef industry is supporting the growing popularity of heat-and-serve products with advertising that targets women of ages 25 to 54 (Henderson, 2001); two television commercials were featured in a three-week advertising burst during February 2001 and were seen by about 51 million women in the target audience. And, research shows that the promotion of the new convenience beef products is having an impact with consumers. According to Gallup tracking polls, awareness of heat-and-serve beef products after an advertising campaign in October 2000 reached 62%, compared with 43% in October 1999 (Henderson, 2001). According to NCBA (Food Systems Insider, 2001a), the number of new products introduced into the retail market in 2000 was: (a) 72 beef heat-and-serve items, (b) 141 poultry products, (c) 58 pork products, and (d) 136 meat substitutes. In March 2001, Albertson's, Kroger, A&P and HyVee were among the first supermarket chains to agree to distribute Farmland Foods' "Ground & Browned™", a fully cooked ground seasoned beef (Dittmer, 2001). David Hall (Farmland Foods) said, "Our research has consistently shown that consumers want simple meal solutions but at the same time they want to maintain the tender and juicy home-made flavor of fresh ground beef...Farmland Foods' Ground & Browned™ does both" (Dittmer, 2001).

And now, the beef industry is even developing new beef products for the international trade. According to Texas Cattle Feeders Association (2001b), the U.S. Meat Export Federation has rolled out "Mega Ball," a cartoon character and a beef product designed to build demand for U.S. beef in Taiwan. The fabricated and pre-cooked meatball, made from the chuck, can be used in popular and

traditional Chinese hot pot dishes and soups. Introduced last year, the product has generated more than $5 million in sales during 2000.

Barkema et al. (2001) reported that consumers are eagerly buying more conveniently prepared food products of consistent quality, despite the sluggish growth of overall food spending. The share of food expenditures that pays for processing, packaging and transportation is climbing, while the share that pays for raw farm commodities is falling, down from nearly a third in 1970 to just a fifth in 1999. Plain (2001) describing "Ten Trends Changing The U.S. Swine Industry" said that, in the past 40 years: (a) farm share of retail pork price dropped from 55% in 1960 to the present 25%; (b) packer share dropped from 29% to the present 17%; and (c) processor/distributor/retailer share increased from 16% in 1960 to the present 58%, revealing that profit potentials in the pork meat business (and probably, in the future, for beef) are in the processing and retailing sectors—in making the product more consumer friendly.

National Cattlemen's Beef Association (NCBA) has sponsored contests to recognize outstanding new consumer-friendly, processed/prepared beef items. In 1998, the NCBA awarded $250,000 to Harris Ranch Beef Alliance for its development and marketing of the "Fully Cooked Beef Pot Roast With Natural Gravy" (Wood, 2000a). In 2000, NCBA awarded $100,000 to RMH Foods, Inc. for "Quick-n-Easy Top Sirloin Steaks In Bourbon Sauce," $100,000 to Kings Command Foods, Inc. for "Old Fashioned Style Beef Patties," $25,000 to Eaves Food, Inc. for "CAB Bubba Burger" and $25,000 to Harris Ranch Beef Alliance for "Fully Cooked Western Style Beef Stew" (Cooper, 2000). National Cattle Women announced in April 2001 (Calf News, 2001) that the National Beef Cook-Off® (to be held in September 2001) will offer prizes totaling $110,000 for winning recipes in each of four categories: (a) fresh beef, (b) prepared beef, (c) fresh beef and fresh potato, and (d) prepared beef and prepared potato; all entries will be judged based on taste, ease of preparation and appearance.

Wood (2000a) described the Brand Mark ("Mark Of Beef Quality") by saying "It tells customers that the product delivers all the things consumers expect from beef—taste, quality and satisfaction. More than 30 beef products are now approved to carry the beef industry's Brand Mark." Companies allowed to use the "Mark of Beef Quality" on their packages and in their advertisements include Burnett & Sons, Mosey's, No Name Steaks, Hormel, RMH Foods, Louis Rich and Stouffers. Rocke (2001) described the essential elements of RMH Foods, Inc. preparation of Meal Solutions (refrigerated, ready-to-eat, center-of-the–plate entrées) as follows: (1) raw material selection—high quality, always; (2) portioning—retailers have forced fixed weights and fixed unit pricing; (3) seasoning—RMH Foods, Inc. wants a unique flavor signature (e.g., the use Wild Turkey bourbon or tequila to flavor two of their signature products) and they try to use robust flavors to bridge from ready-to-eat products to restaurant-quality foods; (4) prepackaging, then cooking—to assure food safety by minimizing post-pasteurization bacterial contamination and to assure 90 days of refrigerated shelf-life; (5) cooking and chilling—cooking is done "low and slow" (e.g., 13 h for their pot roast) and in an essentially air-free atmosphere (because it is prepackaged and then cooked) to lessen occurrence of warmed-over flavor in reheated in the home product; and (6) final packaging—the package around the prepackage is used to "tell the story" to the consumer.

To make beef "easy to choose," it has been advantageous for the beef industry to develop an integrated consumer marketing/communication program with the following elements: (a) demand driver is "nutrition;" (b) focus is personal; (c) message is "Beef Is A Healthy Food;" (d) emphasis is on beef's contribution to the diet of zinc, iron, protein and B vitamins; and (e) strategies and tactics include advertising, communication with health influencers, public relations, education and interactive marketing (Thomas, 2000). NPD/National Eating Trends (2001) reported that: (a) it's easier to change our attitude than our behavior…Americans are becoming more tolerant about being

overweight; 55% of homemakers agreed that "People who are not overweight look a lot more attractive" in 1985 but only 24% agreed in 2000; (b) homemakers are less concerned about calories in meals (39% were in 1990, 25% were in 2000) and about serving foods with fats (51% were in 1994, 33% were in 2000) than ever before; (c) 39% in 2000, as compared to 62% in 1990, of women agreed that "I carefully plan my household meals to be sure they are nutritious;" and (d) 56% of homemakers agreed that "Beef is okay as long as you have a well-rounded diet" and 46% of homemakers agreed that "Beef provides important nutrients to kids'/teens' diets" but only 23% of homemakers agreed that "Beef is higher in nutrients (iron and zinc) than chicken" and the majority (more than 77%) were not aware of beef's zinc and B-vitamin benefits or that beef helps their immune system.

The Vision Statement of the National Cattlemen's Beef Association (Thomas, 2000) is "A dynamic and profitable beef industry, which concentrates resources around a unified plan, consistently meets consumer needs and increases demand." Olson (2000) said "Despite the nutritional attributes of beef, convenience and versatility are the primary drivers that sell it, according to the NPD Eating Trends Survey, while price, taste and nutrition are secondary drivers." Unquestionably, the strategy NCBA has followed in using market research to promote development of new, consumer-friendly beef products has increased demand for beef in the U.S.

Conclusions

Beef lost market-share spending to poultry from 1980 to 1998, in part because the poultry industry was so adept at developing and merchandising processed/prepared products that fit consumer needs for convenience. During that period (1980 to 1998), the beef industry recognized that it must imitate its most formidable competitor—the poultry industry—in identifying demand drivers and concentrating its resources by re-inventing itself and becoming consumer-driven rather than production-driven. Market research on food selection concerns, on shopper's eating habits, on sources of takeout foods, on consumer eating trends and on food trends, as such research related specifically to both fresh (unprocessed) and processed/ prepared beef products, has been capitalized upon and reacted to by the beef industry in the U.S. Household incomes have climbed and household schedules have tightened; so, consumers prefer more conveniently prepared food products and are willing to trade a portion of their bigger disposable incomes for scarce discretionary household time. "Meal Solutions," generated via value-added processing, are necessary because preparation of a meal requires time and knowledge. Time-conscious consumers and those who don't know how to cook seek take-home meals and home meal replacements. Among the demand drivers, "convenience" and "nutrition" surfaced as the two elements of greatest concern to the targeted segment of consumers—women, 25 to 54 years of age, with children at home. Because that audience (59 million people) has a strong need to satisfy and take care of their family, feels that time spent with the family is a priority, may know how to cook but lacks the desire to spend time cooking, and doesn't want to waste valuable time or energy on meat decisions, "convenience" and "nutrition" were apt descriptors for properties in a food product that might satisfy the wants and needs of those targeted consumers. Activities of the National Cattlemen's Beef Association, using beef checkoff dollars, have included the "Beef Must Be EZ To Use; Beef Must Be EZ To Choose" campaign; the "Beef Made Easy" supermarket labeling program; the "Muscle Profiling" research study; the R&D Ranch affiliate development of new convenient beef products; contests to recognize outstanding new consumer-friendly, processed/prepared beef items; development and industry use of the "Mark of Beef Quality," and; the "Beef Is A Healthy Food" campaign. Industry has also responded to market research by developing programs to make purchases of fresh beef easier for consumers (e.g., HEB Supermarkets) and by developing consumer-friendly, value-added and ready-to-eat beef products (e.g., Harris Ranch Beef Alliance; Certified Angus Beef). Unquestionably, the strategy followed by the National Cattlemen's Beef Association in using market research to promote the development of new, consumer-friendly beef products has increased demand for beef in the U.S.

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Table 1. Factors "very important" in food selection.

Item	1988	1992	1996	1998	2000a
Taste	88%	89%	88%	89%	89%
Nutrition	72%	77%	78%	76%	71%
Product safety	83%	71%	75%	75%	71%
Price	65%	75%	66%	64%	69%
Storability	53%	46%	43%	45%	46%
Food preparation time	ND	41%	38%	36%	37%
Ease of preparation	39%	36%	36%	37%	36%
Recyclable packaging	ND	45%	34%	31%	29%

ND = not determined

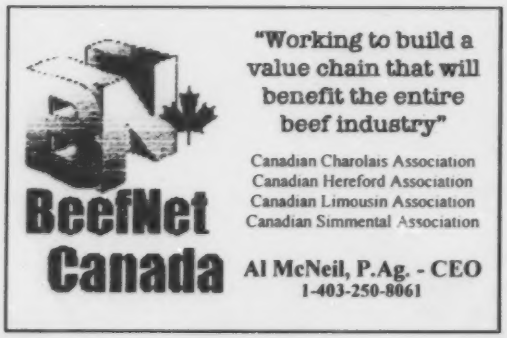
Table 2. How often consumers do certain things relative to eating habits.

Characteristics	1997	1999	2001
Eat home-cooked meals at home	87%	88%	83%
Serve meals using leftovers	43%	41%	38%
Dine out at full-service restaurants	24%	25%	21%
Eat out at fast-food establishments	20%	20%	19%
Eat meals at home that are not prepared at home, like takeout or home delivery	15%	15%	19%

Table 3. Where consumers purchase meals eaten, but not prepared, at home.

Item	1988	1996	2001
Fast-food restaurant	41%	48%	32%
Restaurant	38%	25%	23%
Supermarket	11%	12%	20%
Deli/pizza parlor/shop	ND	4%	5%
Gourmet or specialty store	ND	3%	3%
Convenience store	ND	1%	1%

ND = not determined



Session VI - Animal Behaviour

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Speakers:

Dr. Gerry Mears
Dr. Al Schaefer

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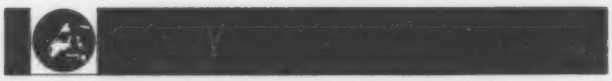

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Assessment of Stress in Cattle from Standard Management Practices in Beef Production

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Key Points

- Stress has an impact on cattle, ranging from apprehension to reduced production and reproduction, compromised health, and even death.
- An objective means of assessing stress to cattle at all levels of production is necessary to assure the consumer that meat animals are raised in a welfare friendly manner.
- An objective means of assessing stress needs to be developed as soon as possible to allow the beef industry to be proactive in addressing animal welfare concerns.

Introduction

Economic losses associated with stress in cattle continue to be a major cost to the beef industry. The negative effects of standard management practices, including weaning, novelty of relocation, changes in diet, transport, lairage, mixing, handling and processing (vaccination, castration, ear tagging, branding, etc.), contribute to reduced growth and reproductive performance, greater health and disease problems, and reduced carcass quality. Epidemiological studies have indicated that there is an association between stressful events in calves and subsequent losses due to infectious diseases (Andrews, 1976). Altered endocrine and neuroendocrine status as a result of stress lead to immunological and reproductive problems (Moberg, 1987) as well as dark-cutting meat and greater shrink in carcasses (Schaefer et al., 1990). Part of effectively reducing the negative impact of stressful practices is identifying which are the most adverse for the animals. Unfortunately, we do not have a generally accepted method of objectively measuring stress in cattle (Rushen and de Passillé, 1992). The ability to do this would allow producers to cut production costs and losses associated with stress by altering management practices to minimize stress. Furthermore, having an acceptable means of assessing stress would allow cattle producers to be proactive in identifying and rectifying practices detrimental to animal welfare, cutting short misguided welfare claims that are becoming more prevalent each year. Animal well-being is an issue that is of growing importance to the livestock industry (AFAC, 2001). Animal welfarists have raised society's concern about the well-being of farm animals, and animal well-being has become an issue, with the public asking questions of the cattle industry. As a result of public outcry, recent European farm animal welfare legislation has resulted in policies that restrict animal production practices. Unless proactive steps are taken, it is only a matter of time before similar animal welfare legislation occurs in Canada.

Standard management practices employed in cattle production systems can cause differing degrees of stress. Cattle respond to these stressors behaviorally and physiologically. The response is complex, much of which we do not fully understand. Behavioural responses are fairly evident to

producers and those familiar with animal behaviour, but are difficult to evaluate and quantify. This is due in part to the huge differences in the behavioural response to stress between individual animals, as well as the fact that each observer sees the responses differently. To overcome this, animal behaviourists have developed equipment and protocols to objectively assess the behavioural responses to stress (Schwartzkopf-Genswein et al., 1997; 1998). The techniques allow for the quantification of animal responses to a variety of different stressors and have been invaluable tools in behavioural research.

A range of physiological responses to stress occurs. Body temperature (BT) and heart rate (ECG) may be useful physiological indicators of stress in cattle. While it is clear that cortisol levels are elevated in response to stress, there is disagreement as to whether or not the elevation is proportional to the degree of stress. Despite this, cortisol is the best physiological indicator of stress in cattle that we have at the present time. The neuropeptide, β-endorphin, is an opioid peptide released by the pituitary that acts as a pain killer, which may also be an indicator of stress. Initial studies indicate that sheep respond with elevated plasma β-endorphin following tail-docking and castration (Shutt et al., 1987), and skin pinching and isolation (Hashizume et al., 1994). Dairy cows respond with elevated circulating levels of β-endorphin when placed in an unfamiliar room for milking (Bruckmaier et al., 1993).

Most research on the stress imposed by routine management practices in cattle production has been done by animal behaviourists and animal physiologists working independently. We have both animal behaviourists and physiologists at the Lethbridge Research Centre, giving us a unique opportunity to combine both of these disciplines in the search for objective means of assessing stress in farm animals. Identifying adverse, stressful management practices will enable producers to improve animal health, increase production efficiency, and be proactive in addressing animal welfare concerns. The increasing concerns of society about animal welfare in livestock operations may have major negative implications for beef production if they are not addressed satisfactorily.

The objective of this presentation is to discuss our research to develop means of objectively assessing stress caused by husbandry practices used in beef cattle production. It is important that the cattle industry press forward with animal stress and welfare research as it will take several years to develop these procedures and to assess the stress induced by standard husbandry practices.

Discussion

Physiological Responses to Physical and Psychological Stressors in Lambs

Our initial studies were done with lambs to develop and evaluate procedures for obtaining hormone responses to stressors (Mears and Brown, 1997). Plasma cortisol, β-endorphin, triiodothyronine (T3) and thyroxine (T4) concentrations were determined in lambs before, during and after exposure to stress in order to evaluate the potential use of these hormones to objectively measure stress responses. Lambs were exposed to two physical stressors, tail docking and castration, and three psychological stressors, weaning, isolation and restraint. Twelve ewe and 24 ram lambs were assigned to the experiment, with 12 of the ram lambs castrated at 3 wk of age.

Plasma T3 and T4 concentrations were not affected by any of the stressors imposed on these lambs despite reports in the literature that acute stress causes rapid, but transient increases in plasma concentrations in cattle (Scott et al., 1993) and hogs (Farmer et al., 1991). Our sampling schedule should

have allowed us to detect any increases in T3 and T4 following stress, unless plasma concentrations returned to baseline levels within 15 minutes of exposure to the stressors tested.

Tail docking with an electric hot iron tail docker within 24 h of birth did not alter either plasma β-endorphin or cortisol (Table 1). The levels of these hormones are elevated at birth and remain so for the next 24-48 h, which may mask any effects of the tail docking at that time. Certainly, tail docking in older lambs (2-6 wk old) elevates both plasma β-endorphin and cortisol (Shutt et al., 1987).

Surgical castration of 3 wk old ram lambs resulted in large elevations in plasma β-endorphin and cortisol concentrations (Figures 1a and 2a). β-endorphin levels were still elevated 24 h after castration, whereas cortisol levels returned to baseline levels between the 3 h and 24 h samples. Clearly, these data indicate that surgical castration causes a marked distress in lambs for at least 24 hours following castration.

The three psychological stressors caused varying responses in plasma β-endorphin and cortisol. Weaning did not affect plasma β-endorphin (Figure 1b). Weaning elevated plasma cortisol, but to a much smaller degree than for castration, and for only the first 60 min, and again at 24 h (Figure 2b). Thus, while the lambs were observed to vocalize their displeasure at being separated from their mothers, weaning caused only a minimal hormonal response.

Isolation caused small, but significant elevations in plasma β-endorphin (Figure 1c) and cortisol (Figure 2c) during the initial 60 min. This coincided with the duration of isolation and was intermediate to the response obtained for castration and weaning.

Restraint (designed to mimic shearing restraint) for 4 min resulted in small increases of short duration for both hormones. Plasma β-endorphin was elevated only at 7 min (Figure 1d). Plasma cortisol was elevated for only the first 30 min after restraint was initiated (Figure 2d). The magnitude of cortisol and β-endorphin changes following restraint suggest that restraint was a moderate stressor in these lambs.

This study clearly showed that surgical castration had a much larger effect on plasma β-endorphin and cortisol profiles than the psychological stressors of weaning, isolation and restraint. Plasma cortisol responses to psychological stressors were more pronounced than β-endorphin responses. The psychological stressors resulted in graded responses for both hormones in the following order: isolation > restraint > weaning, which may indicate the relative stressfulness to the animals of each of these husbandry practices. The results indicate that plasma β-endorphin and cortisol profiles obtained during stressful events may be of use in assessing the relative stressfulness of these events to lambs.

Physiological Responses to Handling, Shearing and Previous Shearing of Ewes

We next conducted a study to determine if shearing stressed sheep over and above the stress resulting from the handling associated with shearing (Mears et al., 1999). We also wanted to know if sheep exposed to previous shearing responded differently to shearing than sheep that had never been sheared. Plasma β-endorphin and cortisol concentrations were determined during either handling only, or handling plus shearing, in 12 ewes previously exposed to shearing (1-4 times) and in 12 naïve ewes. Half of each group were sheared and the other half were sham sheared to simulate shearing without the shears actually touching the ewe.

Handling and shearing resulted in large increases ($P < 0.0001$) in plasma β-endorphin and cortisol concentrations (Tables 1 and 2). There were, however, no differences ($P > 0.1$) in plasma β-endorphin and cortisol responses to either handling alone, or handling plus shearing. Therefore, shearing did not stress ewes beyond the cumulative stress resulting from the handling procedures.

There was a big difference in the development of the β-endorphin and cortisol responses to shearing and handling in this study. Simply moving the sheep from the sheep barn to the shearing platform right next door was sufficiently stressful to affect plasma cortisol levels (Table 3, 0 min). Plasma β-endorphin, however, was not elevated until after shearing was completed (Table 2, 7 min). There was also a major difference in the duration of the hormone responses. Plasma cortisol remained significantly elevated for the entire sampling period of 60 min (Table 3), whereas β-endorphin concentrations wee only elevated between 7 and 15 min after shearing (Table 2).

Prior exposure to shearing enhanced both the β-endorphin ($P < 0.05$) and the cortisol ($P < 0.001$) responses to shearing and handling, compared to naïve ewe responses (Tables 2 and 3). The experienced ewes responded with elevated β-endorphin levels as soon as they were moved to the shearing platform. This lasted for over 30 min. By contrast, naïve ewes had only a transient plasma β-endorphin response to shearing and handling at 7 min, and by 60 min actually had lower plasma β-endorphin levels than they had before shearing began. Similarly, plasma cortisol responses to shearing and handling remained much higher throughout the experiment in ewes previously exposed to shearing, whereas they returned to pre-shearing levels by 60 min in naïve ewes.

We were surprised by the apparent negative adaptation to previous shearing exposure indicated by these hormone results. Our casual observations during the experiment indicated that the previously sheared ewes appeared less upset with shearing and handling than the naïve ewes, leading us to expect smaller β-endorphin and cortisol responses in the naïve ewes, instead of the larger responses we observed.

The results of this trial further convinced us that evaluating profiles of β-endorphin and cortisol responses to stress would be useful in assessing stress in sheep. The apparent negative adaptation in experienced ewes, which appeared to contrast with our casual observations of their behaviour, emphasized the need to include a behavioural component in our studies.

Physiological and Behavioural Responses to Standard Husbandry Practices in Cattle

Two years ago we began a series of experiments designed to combine the assessment of both behavioural and physiological responses to standard husbandry practices in the cattle industry. Our goal is to use the responses obtained to develop an objective means of assessing the relative stressfulness of these practices to cattle. In the first year we adapted for use with cattle, the techniques we had developed for measuring stress in sheep. Dairy calves were exposed to castration, dehorning, and restraint. We measured plasma cortisol, plasma β-endorphin, body temperature and heart rate before, during, and after exposure to the stressor. Behavioural responses such as tail flicking, vocalization and kicking were also observed and recorded.

During the second year we moved our experiments to beef cattle. Calves were exposed to transportation, restraint, mixing with strange cattle, castration and branding. Responses were determined before, during and after exposure to these stressors. Plasma cortisol, plasma β-endorphin, blood packed cell volume, body temperature and heart rate were determined. The behavioural responses of tail

flicking, vocalization, kicking, escape attempts, lying down, standing, walking, confrontation, ruminating, drinking and eating were collected. We are now in the process of analyzing this data and preparing it for publication.

We recently reported the transportation component of these studies (Booth et al., 2001). Nineteen finished steers underwent a short haul, 3 h round trip of 258 km. The trip was on a divided highway that passed through seven towns/cities. There was no mixing with strange animals during transportation. Behavioural observations were obtained on the day prior to transportation for 1 h corresponding to the projected time of the first hour after transportation on the day of transport. Animals were fitted with jugular cannulas and heart rate monitors on the morning of transport. Blood samples were obtained immediately before loading, at unloading, and 1 h after unlading. Heart rates were continuously monitored from 1 h prior to loading until 1 h after unloading. Behaviour was monitored for 1 h prior to loading for transport and for 1 h after unloading.

Plasma cortisol concentrations were higher ($P < 0.05$) at unloading after 3 h of transport than they were just prior to loading for transport (Table 4). One hour after unloading, plasma cortisol levels had dropped considerably from the levels found at unloading ($P < 0.001$), and were numerically lower than they were before loading. Heart rates were markedly influenced by the events that the steers were exposed to (Table 5). Loading resulted in over a 50% increase ($P < 0.001$) in heart rate compared to the heart rate found in standing animals prior to loading. Heart rates were also elevated ($P < 0.05$) during travel on highways and in town, and at unloading when compared to pre-loading heart rates. Heart rates remained elevated ($P < 0.05$) in standing animals during the hour after unloading, but dropped to pre-loading rates in animals that were lying after unloading. Comparison of heart rates for standing and lying animals during the hour after unloading with those of standing animals prior to loading is of particular interest. There are energetic costs to the animals of maintaining balance in the moving vehicle during transportation. The elevated heart rates in standing animals compared to lying animals in the hour after unloading would seem to indicate that the animals were physically tired after their trip. The behavioural record appears to confirm this in that steers spent a greater amount of time lying down and a lesser amount of time standing after transport than they did at the same time the day prior to transport (Table 6).

The average time that each steer spent eating following transportation was similar to the time each spent eating at the same hour on the day prior to transport (Table 6). However, the number of animals that ate during the first hour after unloading was almost double the number that ate at the same time the previous day (Figure 3). Similarly many more steers drank water and laid down following transport compared to the day prior to transport (Figure 3). Surprisingly, rumination appeared to be unaffected by transport.

Thus, the physiological and behavioural data collected under the conditions of this study indicate that a short haul journey of 3 h was stressful to finished steers, despite the fact that they were used to being handled. Although cortisol levels returned to pre-transport levels within 1 h of unloading, both the elevated heart rate and the behavioural responses of the animals suggest that they were physically tired by the journey.

Implications

Developing an objective means of assessing stress is imperative if the beef industry is to be proactive and improve animal welfare in production systems. Our research has laid the foundation for

developing a protocol to assess stress in cattle. We are continuing to work in this direction, but it will take more research and time to achieve this. The greater the support and financial assistance that the beef cattle industry supplies researchers with, the sooner we can assure the public that the beef they are consuming is raised in a welfare friendly manner.

Acknowledgments

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Table 1. Effects of tail docking on plasma β-endorphin and cortisol concentrations (mean ± SE)[z]

Sampling time	β-endorphin (pmol/L)	Cortisol (μg/dL)
"0" min[y]	82.5 ± 6.6	6.3 ± 0.6
15 min[x]	88.6 ± 8.7	8.0 ± 0.6
30 min	79.2 ± 6.4	7.5 ± 0.6
1 h	76.9 ± 5.8	6.4 ± 0.6
3 h	83.3 ± 8.3	5.4 ± 0.6
24 h	68.4 ± 5.2	4.9 ± 0.4

From Mears and Brown (1997).
[z] $n = 36$ lambs per mean.
[y] Sampled immediately before tail docking.
[x] Time sampled after tail docking.

Table 2. Effect of treatment (sham *vs.* sheared) and prior exposure to shearing (naïve *vs.* experienced shearing 1-4 times) on mean plasma β-endorphin concentrations (pmol/L)[z]

	Treatment (Trt)		Prior exposure (PE)			Probability	
Sample	Sham	Sheared	Naïve	Experienced	SE	Trt	PE
Pre[y]	34.1	28.8	32.5	30.5	3.31	NS	NS
0[x]	41.4	32.9	30.3	44.0*a*	4.75	NS	0.0555
7	53.3*b*	47.1*b*	46.6*a*	53.8*c*	4.87	NS	NS
15	46.1*a*	38.4	32.9	51.6*c*	4.28	NS	0.0059
30	34.5	34.3	27.1	41.7*a*	3.51	NS	0.0078
60	28.2	25.2	19.8*a*	33.6	3.15	NS	0.0055

From Mears et al. (1999).

[z] $n = 12$ ewes per mean.

[y] Pre-shearing sample taken prior to moving sheep into shearing room.

[x] Sampling time (min) relative to start of shearing or simulated shearing.

a-c Different from pre-shearing mean within the same column (*a*, $P < 0.05$: *b*, $P < 0.01$; *c*, $P < 0.001$).

Table 3. Effect of treatment (sham *vs.* sheared) and prior exposure to shearing (naïve *vs.* experienced shearing 1-4 times) on mean plasma cortisol concentrations (μg/dL)[z]

	Treatment (Trt)		Prior exposure (PE)			Probability	
Sample	Sham	Sheared	Naïve	Experienced	SE	Trt	PE
Pre[y]	2.1	2.7	2.1	2.6	0.40	NS	NS
0[x]	6.2*c*	5.2*b*	3.9*a*	7.6*c*	0.75	NS	0.0026
7	7.2*c*	6.8*c*	5.3*b*	8.8*c*	0.76	NS	0.0038
15	7.7*c*	7.6c	5.8*c*	9.5*c*	0.65	NS	0.0006
30	7.5*c*	7.3*c*	5.0*c*	9.8*c*	0.63	NS	0.0001
60	5.9*c*	5.9*c*	3.7	8.1*c*	0.78	NS	0.0006

From Mears et al. (1999).

[z] $n = 12$ ewes per mean.

[y] Pre-shearing sample taken prior to moving sheep into shearing room.

[x] Sampling time (min) relative to start of shearing or simulated shearing.

a-c Different from pre-shearing mean within the same column (*a*, $P < 0.05$: *b*, $P < 0.01$; *c*, $P < 0.001$).

Table 4. Effect of 3 h of transportation on mean plasma cortisol concentrations of finished steers[z]

	Pre-transport	Post-transport	Post-transport + 1 h
Plasma cortisol (ug/dL)	1.87	3.10[y]	1.16[x]
SE	0.28	0.33	0.20

From Booth et al. (2001).

[z] $n = 16$ steers per mean.

[y] Post-transport cortisol is greater than pre-transport cortisol ($P < 0.05$).

[x] Post-transport + 1 h cortisol is less than post-transport ($P < 0.001$).

Table 5. Effect of 3 h of transportation on mean heart rates (bpm) for finished steers[z]

	Pre-transportation		Transportation			Post-transportation	
	Standing	Loading	Highway	In town	Unloading	Standing	Lying
Heart rate	76.3*a*	119.9*d*	84.9*b*	90.3*bc*	95.6*c*	87.7*bc*	70.3*a*
SE	1.8	4.4	4.1	3.6	6.4	2.9	2.2

From Booth et al. (2001).

[z] $n = 9$ steers per mean.

a-c Numbers in row with different letters are significantly different ($P < 0.05$).

Table 6. Effect of 3 h of transportation on time spent (mean ± SE) standing, lying down and eating by finished steers[z]

	Standing[y]	Lying down[y]	Eating[y]
Day prior to transport	46 ± 4	12 ± 3	1.8 ± 0.6
One h post-transport	27 ± 2	33 ± 2	3.2 ± 1.1
P	< 0.05	< 0.05	NS

From Booth et al. (2001).
[z] $n = 19$ steers per mean.
[y] Time spent in min.

Non-Invasive Assessment of Stress

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Key Points

- Stress can have a significant impact on animal growth and productivity, carcass yield, meat quality and safety, predisposition to disease susceptibility and general welfare.
- Understanding the nature and time course of stress factors is the first step towards managing such stress in animals. Unfortunately, most conventional methods for assessing stress are in themselves stressful.
- The use of non-invasive measures of stress, notably infrared thermography and salivary cortisol present a unique opportunity to study and manage stress in cattle to provide an earlier detection of stressed animals.

Introduction

Cattle producers spend a substantial amount of time and resources treating animals for a host of disease conditions. Estimates vary from $10 to $25 for average direct treatment or processing costs and that does not include the added cost of reduced productivity, carcass yield loss and reductions to beef quality for animals that become ill. A reasonable question thus arises regarding why these conditions exist and what might be done about them to improve such factors as animal production efficiency, beef quality and animal welfare.

An examination of these events clearly indicates that animal stress is a predisposing and underlying factor affecting these aforementioned events. Thus, a study of animal production and beef quality is to a large extent a study of animal stress.

Definition of Stress

A number of models have been proposed to describe the term stress (Hubbard and Workman, 1997). The most common model used in animal science is a biological model in which known endocrine measurements can be quantified. The concept of stress and the use of the term is often attributed to a Canadian researcher by the name of H. Selye (1956). Selye described stress broadly as "the wear and tear of life" and any factor that caused an increase in a specific endocrine product called cortisol. Cortisol is a hormone produced by adrenal glands when the brain (hypothalamus and pituitary) signals the adrenals to release it. This is the so-called HPA axis definition or Hypothalamus-Pituitary-Adrenal model.

Why is Stress Important?

Stress can influence virtually every organ system of an animal's body both in terms of physiological stress and cognitive events (Hubbard and Workman, 1997). Some very interesting reviews on the subject are available (Gross and Siegal, 1993; Saploski, 1994). In terms of animal sciences, stress, or events which cause an increase in circulating cortisol, can lead to a host of

problems including disease predisposition (Hubard and Workman, 1997; Balm, 1999), reduced animal growth and productivity (Stephens, 1980), reduced carcass yield (Scott et al., 1993; Schaefer et al., 1997; 2001), reduced meat quality (Schaefer et al., 1997; 2001) and degraded animal welfare (Fraser, 1979; Lefcourt, 1986; Grandin, 1995).

Assessment of Stress

The measurement of stress originally involved such indicators as the weight of adrenal glands or an estimate of so called LD 50 (lethal dose 50) for animals in a given situation. More recently, however, a number of alternative measures have been used including hematology (Murata, 1989; Schaefer et al., 1992; Church et al., 1994), enzymatic factors (Scott et al., 1993; Schaefer et al., 1997), the monitoring of blood or tissue metabolites (Lacourt and Tarrant, 1985; Schaefer et al., 1990), immunology factors (Blecha et al., 1984), endocrine factors (Fordham et al., 1989; Scott et al., 1993). Blood gas and acid-base/electrolyte (Schaefer et al., 1994), biochemical indicators (Roberts et al., 1996), meat yield and quality (Schaefer et al., 2001) and acute phase proteins (Schaefer et al., 2000) to mention a few. However, as discussed by Sinatra et al. (1992) no one measurement of stress is perfect.

The Quandary

An ideal measurement of stress would be one that was non-invasive or in other words did not require the capture, restraint and blood sampling of an animal since this very act induces stress. Secondly, an ideal measurement would also act as an early indicator of stress. Unfortunately, all of the aforementioned measurements, while showing utility, nonetheless require the capture, restraint, manipulation and/or blood sampling of the animal. The consequence of these measurements is that the very act of collection to some degree biases the outcome. Furthermore, many of these measurements only display changes once a stress or biological condition has already been manifest. In other words, one is looking at history, not predictors of future events.

Non-Invasive Stress Measurement Options

The original non-invasive measurement of stress in an animal is simply the study of animal ethology or behaviour (Hafez, 1969; Kilgour and Dalton, 1984). Animals in distress typically display revealing characteristics, which can be used to assess the impact of their environment. However, measures of animal behaviour are often subjective. Moreover, prey species and animals such as cattle can disguise their behaviour so as not to display abnormalities to predators. Hence, an assessment of animal behaviour may not be as quantitative as desired.

Other attempts to non-invasively or less invasively measure stress in animals have included the use of remote or self contained blood sampling equipment for use in animals on pasture (Matthews, 2001) and the implementation of various forms of electronic identification systems (EID). The use of EID in particular has been interesting. Original units suffered from symptoms of bulkiness, short battery life, incompatibility with available computer reading systems and limited data storage capacity. However, the current use of passive transponders is showing considerable promise (McAllister et al., 1999) enabling beef producers to remotely obtain information regarding the whereabouts of an animal, it's feed and water consumption as well as other behaviour. In fact, some systems are even being linked to Global Positioning Satelites (GPS) (Turner et al., 1999). However, again, these systems, while very useful, lack the objective and specific ability to monitor stress per se or at the very least, require a time course of information before an animal in distress can be verified.

A further device used to non-invasively monitor stress in humans is the orthodontic dental sink or ODS devise (Wade and Haegle, 1991). In essence, this devise consists in principle of a small, pea sized, plastic bead that can be attached to a tooth. The bead is appropriately prepared or coated with an antibody for cortisol and hence absorbs salivary cortisol over a specific time frame during which the bead is in the mouth. As such the device can thus be used to monitor a total cortisol response to an event. However, the application of this device in domestic animals is as yet theoretical.

Infrared Thermography

Animals emit energy in many wavelengths of the electromagnetic spectrum. This energy emission is often reflective of the metabolic state and stress state of the animal. Infrared thermography is simply the measurement of emitted energy typically in the 5-15 micron wavelength range of the electromagnetic spectrum. Infrared images are typically collected with an infrared camera at a distance from the animal.

Infrared energy from animals represents between 40-60% of the heat energy lost by an animal (Kleiber, 1975). As shown in Figure 1, changes in the infrared image displayed by a beef animal are consistent (correlated) with changes in the animal's objective stress indicators such as cortisol. Therefore, infrared can be used as a non-invasive image technique to monitor stress in animals. In our lab, infrared has been used in a variety of circumstances to monitor stress in cattle. These situations include the detection of transport and handling stress in cattle (Figure 1) as well as the detection of cattle predisposed to producing Dark-Firm and Dry (DFD) beef (Tong et al., 1995; Figure 2), the early detection of cattle displaying a viral infection (Schaefer et al., 2000; Figure 3), and the detection of cattle showing a response to endotoxin (Scott et al., 2000).

The authors note with interest that in the case of Bovine Viral Diarrhea (BVD type 2, Figure 3) infection in calves that the infrared indicated animals displaying infection several days to one week earlier than conventional clinical signs. Obviously, such early detection would enable producers to find and treat suspect animals. Such early treatment in all likelihood would be more effective resulting in reduced expense for medication, faster recovery of animals and reduced promotion of antibiotic resistant bacteria. This data is consistent with infrared information collected on receiver calves in an actual feedlot (Figure 4). As shown in Figure 4, calves with a greater degree of stress clearly indicate differences in infrared measures demonstrating both an improved accuracy and sensitivity of the infrared technique over conventional measures such as rectal temperatures.

Salivary Cortisol

As discussed earlier, the stimulation of an animal's hypothalamic-pituitary-adrenal axis (HPA axis) causes the release of the stress hormone cortisol from the adrenals. Cortisol is released into the blood and measured increases in circulating cortisol are assumed to represent an increase in stress. However, the measurement of circulating cortisol presents two problems. Firstly, in order to measure blood cortisol the animal must normally be captured, restrained and blood sampled. Again, this process in itself is a stressor and can bias the results. Secondly, the cortisol contained in blood is often bound to a cortisol binding globulin and thus the true "free cortisol" which is reflective of a stress, is thus masked.

The measurement of cortisol in saliva, rather than blood, largely overcomes some of these difficulties. Firstly, it is usually much easier to simply take a salivary swab from an animal like a cow than it is to restrain and blood sample the animal. Secondly, the cortisol contained in saliva is truly the "free" or unbound cortisol and as such is more representative of the stress state of the animal. The

use of salivary cortisol has thus been employed in our lab. The development of a radioimmuno assay for cortisol has been described by Cook et al. (1996, 1997). Again, data shown in Figure 1 depicts the use of salivary cortisol and its relationship with animal stress.

Prediction Index

Stress is a very complex phenomenon. Therefore, it is unlikely that any one measurement, even a non-invasive measurement of stress, will be totally effective in all situations. The current and future direction taken in our lab is thus to combine measures of stress into an index. For example, one hypothetical approach might be to use the comparatively non-invasive measures of salivary cortisol and orbital(eye) infrared values. The range of values for each parameter could be divided into three ranges as low (lowest third), medium (middle third) and high (upper third). These ranges could be ascribed the values of 1, 2 or 3 respectively. Thus, a stress index for these values could produce a score between 2-6. These scores could then be tested statistically to objectively class an animal with regard to stress response. The use of such indexes in management decisions for example in receiver calf antibiotic programs would have likely application.

Implications

It is important to understand events that affect animal stress because stress can have a significant economic impact on beef production including the predisposition of animals to disease, alterations in carcass yield, meat quality and food safety as well as general growth and productivity. In order to understand animal stress it is necessary to measure it. The present manuscript proposes that the non-invasive measurement of stress is likely to be more revealing, accurate and cost effective. It is further proposed that infrared thermography and salivary cortisol are two methods that show utility in this regard. The practical application of these methods in receiver calves, finished feedlot cattle and in abattoir assessment of animals can be demonstrated.

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Figure 1: Infrared and Salivary Cortisol Changes Pre and post transport

Salivary Cortisol Pre Transport	Salivary Cortisol Post Transport
2.6 nmol/L	5.2 nmol/L $P<0.05$

Lacombe Research Centre data on feedlot cattle transported approximately 3h

Dorsal Infrared Pre-Transport

Dorsal Infrared Post Transport

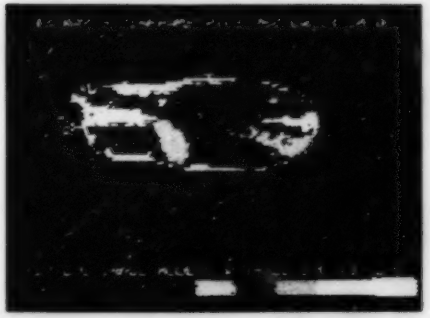
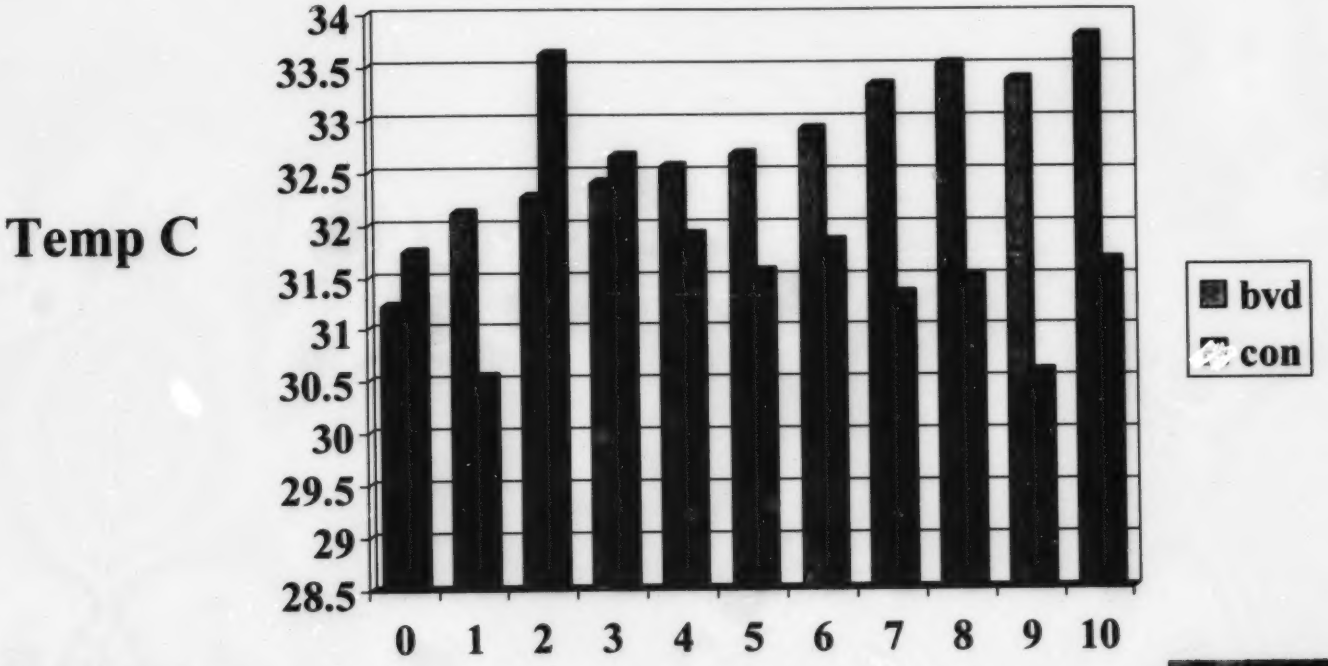


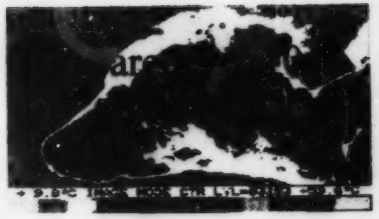
Figure 2: DFD Beef Detection Using Infrared Thermography

Dorsal infrared images of feedlot bulls transported for 4 h. Lacombe Research Centre

From Tong et al., 1995

Figure 3: Orbital infrared temperatures for BVD infected calves





From Schaefer et al., 2000

Figure 4: Infrared (IRT) and rectal temperatures in receiver calves with two levels of stress

	IRT	RECTAL C	
Commercial Calves (multiple source, commingled. Higher stress)	21.6	39.1	n=242
Retained Possession (single source, retained possession. Lower stress)	17.3	39.4	n=296

Session VII - Genetics and Genome Discovery

Sponsored by *Alberta Cattle Commission*

Speakers:

Dr. Denny Crews
Dr. Stephen Moore
Dr. Jim Wilton
Dr. Ron Teather

Genetic Evaluation and Improvement of Economic Merit Using EPD

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Key Points

- Genetic evaluation is a crucial preliminary step toward development of sound genetic improvement programs and has advanced over the past two decades with sophistication in modeling techniques and increases in computing power.
- New traits in genetic evaluation programs will be scrutinized with respect to economic relevance.
- Genetic evaluation and improvement of some economic traits will be accomplished through the use of indicator traits, especially those for which direct measurement is not readily feasible.
- Multiple trait index selection programs will provide powerful improvement tools for economically important traits related to production, reproductive and carcass merit.
- Successful genetic evaluation and improvement programs will result from the combination of both scientific development and field level implementation.

Introduction

Genetic evaluation represents the synthesis of available information into a single value for each animal used for the purpose of ranking prior to selection. The information available for evaluation depends in part on the nature of the trait (e.g., sex limited or not) and in part on the program for data collection (Pollak and Quaas, 1983). In beef cattle, some economically important characteristics, such as weight or rate of gain, can be measured on all animals, thus the potential exists for each animal's evaluation to consist of its own performance record as well as information on the performance of its relatives.

For more than 20 years, genetic evaluation tools in the form of estimated breeding values or expected progeny differences (EPD) have been in use in the beef industry to provide more accurate predictions of the genetic merit of animals with respect to economically important traits. Advances in genetic modeling and increases in computing resources have allowed for genetic evaluation of more animals for more traits. Early sire summaries which reported EPD for basic growth traits (i.e., birth, weaning and yearling weights) have more recently been augmented to include genetic evaluations of entire populations (e.g., breeds) for a wide array of performance characteristics (BIF, 1996).

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Historically, EPD have been estimated for weight traits which have the attributes of relative ease of measurement, and some economic value. Practically, traits for which EPD are estimated should have an economic impact on beef production. In some cases, EPD for so-called indicator traits have been estimated because the related economic trait is difficult or costly to measure (e.g., age at puberty, carcass merit, feed efficiency), or can only be measured later in life (e.g., survival, lifetime productivity, mature weight). Commonly recognized examples of indicator traits (and their correlated economic traits) are scrotal circumference (heifer fertility) and birth weight (post-natal growth rate, calving ease), and most economically important traits have several indicators (Golden et al., 2000).

As EPD methodology continues to mature, the economic relevance of traits must be closely considered, in addition to the diverse range of environments in which selected animals must produce. The use of EPD for economically important traits as selection tools then contributes to a genetic improvement program that can be specific to markets, environments and producers.

This paper will review the development and implementation of genetic evaluation systems and introduce new traits and trait-complexes that would be suitable components of national cattle evaluations (NCE).

Perspectives on National Cattle Evaluation

Accurate prediction of breeding values plays an important role in genetic improvement programs. This is particularly true when relatively few individuals, through high reproductive rate (i.e., artificial insemination), have a major influence on the genetic merit of a population (Kennedy, 1981). Henderson (1963) extended the theoretical justification of selection index and developed a procedure for predicting breeding values, which he later termed best linear unbiased prediction (BLUP; Henderson, 1975), now considered the standard for applied EPD estimation.

The use of genetic evaluation tools such as EPD in genetic improvement is a cumulative process, which can generally be additive and permanent. Rates of genetic progress (i.e., genetic trend) for key economic traits have typically been reported to be in the range of 0.5 to 2.5% of the mean per year (Smith, 1984). In field applications, genetic change in post-natal growth has been significant, as evidenced by a positive genetic trend for both weaning and yearling weights in most breeds. Selection and simulation studies have shown that selection on EPD results in genetic progress, at or near expected rates.

Genetic evaluation has improved tremendously in the past 20 years, mostly due to the development of sophisticated statistical methods that overcome bias inherent to field data (Bourdon, 1998). We can now use these methods, combined with modern computers, to make genetic predictions for more cattle and more traits than ever before. In a recent survey, it was reported that EPD have been estimated for more than sixty different beef production traits around the world (Golden, 2001). The science of genetic evaluation and improvement, however, is far from mature. Bourdon (1998) suggested an integration of genetic predictions with multiple trait selection technology that is usable on a large scale.

New Traits in Genetic Evaluation

Recent research has focused on development of genetic evaluation systems for sets of traits beyond weight and growth rate. There is an emerging trend toward development of EPD for more complex traits, not necessarily directly measurable, using index methods (Bourdon, 1998; Gibson and Wilton, 1998).

In some cases, this is because the trait may be difficult or cost-prohibitive to measure, or cannot be measured until later in life or after a long production period. Conceptually, using experimental data, key components and indicators can be identified which contribute to genetic prediction for a complex trait,

however, successful development of a genetic evaluation system also requires field data to be collected by breed associations and (or) producer groups (Pollak and Quaas, 1983).

Golden et al. (2000) introduced the concept of economically relevant traits (ERT) as a principle and framework to guide the process of identifying traits for which EPD should be produced in the next generation of national cattle evaluations. Several traits, commonly measured on beef cattle and used in NCE programs, do not directly affect profit or risk and are therefore appropriately termed indicators. Because indicator traits are usually easier or more cost effective to measure, but retain high genetic correlation with economic traits of interest, indicator trait EPD are a major component of NCE. Potentially large errors in making selection decisions can result from placing undue emphasis on indicator trait EPD, especially when EPD are available for both the indicator trait(s) and the ERT.

The increased sophistication of both genetic models and the field-level performance recording programs now in place allow for EPD estimation for traits and trait-complexes not previously considered. Most, if not all, of these new EPD can be placed into one of three categories: 1) reproductive merit, 2) carcass merit, or 3) productive life. The typical scenario is for research to be conducted to identify key indicator traits as well as define the economic trait of interest. Next, data collection must be in place in the field to collect information on both the trait of interest and its indicators. Lastly, genetic models are developed which appropriately incorporate data into an evaluation system. To more clearly illustrate, several representative examples will be discussed.

Multiple Trait Index Selection: Maternal Productivity

Definition

Cow or maternal productivity refers to the measurement of outputs in the beef production cycle. The most important output of a cow-calf system is the sale of weaned calves, but income is also derived from salvage of cull cows. Maternal productivity is a complex trait, controlled by the composite effects of cow fertility, cow and calf survival, weaning weight and cow maintenance costs (Mwansa et al., 2001).

The Hereford breed has long been characterized as a maternally-oriented breed. It has been reported that the Hereford cow is of average or better fertility, is moderate in size and milk production, and has the ability to maintain body condition even under extensive management (Gallivan et al., 2000). It is generally accepted that the number and weight of calves weaned in a cow's productive lifetime determines her value in the cow-calf segment. Deland and Newman (1991) argued that cow lifetime productivity is an important consideration in cow-calf operations since costs associated with maintaining breeding animals per progeny produced decrease with increasing numbers of progeny. Therefore, a profitable cow is one that remains in production beyond breakeven and into profitable parities. An organized genetic improvement program for maternal productivity in the Hereford breed was developed at the Lethbridge Research Centre (LRC) and included definition of key component traits and incorporation of those components into an interpretable maternal productivity index (MPI).

Key Components

Component traits were included in the MPI based on their ability and potential to contribute to high weaning weights and consistence of weaning a calf over a prolonged herd life with lower maintenance costs (Mwansa et al., 2001). Three key component traits included in the MPI were: 1) calf weaning weight, 2) cow weight, and 3) cow survival. Conceptually, it is straightforward to understand that calf weaning weight represents the main revenue source in a profitable cow-calf enterprise. Further, survival represents the genetic ability of females to consistently reproduce over a sustained herd life. Finally, cow weight represents the cost associated with maintenance of the cow herd. Since heritabilities (i.e., the proportion of phenotypic differences among animals due to genetic effects) and genetic correlations (i.e., the measure of association among genetic effects) are prerequisite to development of genetic evaluation systems, applicable genetic parameters were estimated for these key component traits using an historical LRC data set involving the Hereford breed (Table 1).

Although data for most components of the MPI can be readily obtained from field and experimental data, few field records would be available for cow weight since this is not a common data component of breed association performance programs. To increase prediction accuracy for this component, a multiple trait approach was taken, with birth, weaning and yearling weights used as indicator traits to predict cow weight. This makes accurate estimation of genetic correlations of cow weight with its indicators essential in this system.

Survival, and alternative measures of longevity can be defined both mathematically and conceptually in several ways. Extending the work of Snelling et al. (1995), we defined survival as the probability that a cow will wean three or more calves given that she became a dam. As a sex-limited trait, survival can only be measured on females and genetic predictions for males are possible through use of advanced models incorporating genetic relationships among all traits and animals.

Component Economic Values and Index Properties

Mwansa and Crews (2001) summarized the economic weights developed for key components of the MPI. To summarize, the complete MPI, constructed as a linear function of component trait EPD and their economic weights, can be written as:

$$MPI = \$3.00 \times EPD_{WWT_D} + \$2.72 \times EPD_{WWT_M} - \$0.49 \times EPD_{COWWT} + \$361.00 \times EPD_{SURV_3}$$

The idea of calculating the relative emphasis on the key component traits is to understand how selection for the MPI would be related to genetic change in the components. Relative importance, calculated by standardizing the economic weights with genetic parameters, was found to be 28:25:16:36, for weaning weight direct, weaning weight maternal (milk), cow weight, and survival, respectively. This implies that genetic improvement in the MPI will be approximately 25% due to genetic change in each of direct and maternal weaning weights, 16% due to change in cow weight, and 36% due to increases in survival (Mwansa and Crews, 2001). By simulation, it was predicted that the genetic trend will be positive (increasing) for all component traits in the MPI, due mostly to the estimated genetic correlations among them (Mwansa et al., 2001). Selection of the top 10% of animals for the MPI would result in positive genetic change of 9.08, 11.57 and 10.06 lbs, in weaning weight direct, weaning weight maternal and cow weight EPD, respectively, and 0.05 in survival, per generation (Mwansa and Crews, 2001). The negative economic weight obtained for cow weight represents a cost, however, due to highly positive

genetic correlation with weaning weight, the genetic trend for cow weight is expected to be positive, although moderated, through correlated response to selection.

Maternal Productivity Index Implementation

Obviously, implementation of an MPI genetic evaluation system at the level of national cattle evaluation requires collaboration between science and industry. Using the field level performance recording programs of the Canadian Hereford Association, EPD for component traits are estimated, leading to their incorporation into a national MPI evaluation. National level implementation must be preceded by research and development using experimental data.

Multiple Trait Index Selection: Terminal Sire Value

Justification

The so-called terminal sire breeds, initially imported from continental Europe, have historically been selected for product traits, especially growth rate and lean meat yield. As an example, the Charolais is a terminal sire breed consistently ranked high for product yield measurements in sire breed comparison studies at the USDA Meat Animal Research Center (L. V. Cundiff, personal communication). Genetic evaluation and improvement of carcass traits has only recently been enabled at the national level through use of organized carcass progeny tests. The standard complement of carcass data includes carcass weight, subcutaneous fat thickness, area of the longissimus (ribeye) muscle and marbling score. The preceding components can be combined to predict retail product or lean yield which can be a composite trait in a genetic evaluation system (Crews and Kemp, 1999). Further augmentation of composite yield measures with marbling score (e.g., as a measure of meat quality) and growth rate have been used to develop a economically weighted multiple trait index with the breeding objective to increase efficient production of lean, high quality beef (Crews, 2000). Using the Canadian Charolais Association Conception to Consumer (CtoC) carcass progeny test and Charolais Herd and Record Management (CHARM) performance databases, a terminal sire value (TSV) index was developed.

Model and Index Development

Following data edits, a total of 3,994 CtoC carcass records were available representing 170 tested sires. Prediction equations for lean yield from the scientific literature (e.g., Crews and Kemp, 1999) were used to predict percent lean yield and lean carcass weight. Heritabilities for component traits ranged from 0.20 to 0.34 (Table 2), similar to summaries of parameters from field data (Koots et al., 1994a,b). Lean growth rate measures were positively correlated with carcass weight and ribeye area, and negatively correlated with measures of fat. Selection for lean yield was shown to have the least expected impact on other composite traits with an efficiency of 0.75 or lower relative to expected response from direct selection on component traits. The CHARM program had far greater numbers of records with fewer records per sire and contemporary group. Sire EPD rank correlations were moderate to high indicating favorable agreement between evaluations based on CtoC and CHARM databases (Caron and Kemp, 1999).

An optimum index was identified based on its desirable pattern of selection response among traits in the objective. The index (TSV), consisting of a linear combination of economically weight EPD was

$$TSV = EPD_{BWT} + 1.5\,EPD_{WG} + 1.5\,EPD_{PWG} + 3\,EPD_{LY} + 2\,EPD_{MAR}$$

where BWT, WG, PWG, LY and MAR, represent birth weight, preweaning gain, postweaning gain, lean yield and marbling score, respectively (Crews, 2000).

Index Efficiency Characteristics

Overall, 70% of the economic gain with this index in achieved through improvement in carcass traits (LY and MAR) with growth traits accounting for about 23%. Less than 7% of the gain originates from changes in BWT. Further, the TSV was found to be relatively robust to changes in economic values and genetic parameters (Caron and Kemp, 1999). The use of a selection index is desirable because it allows for maximization of overall selection response, or composite genetic merit. Potential genetic antagonisms are moderated through the use of multiple trait selection index procedures, and in this case, a majority of economic improvement is through carcass traits in optimal combination (Crews, 2000).

Carcass Merit Prediction

The collective of individual carcass traits related to yield and quality have been termed carcass merit. An increasing research effort has recently been directed toward genetic evaluation and improvement of carcass merit (Bertrand et al., 2000). Both across breed variation and heritability estimates for most carcass traits have been characterized as at least moderate, indicating that selection for carcass traits would be effective given appropriate breeding objectives. However, collection of carcass data suitable for genetic evaluation and improvement programs, especially at the national level, is not trivial. The use of indicator traits and multiple trait evaluation procedures are becoming commonplace for carcass merit genetic improvement programs.

Carcass Merit and Growth

It has been suggested that increasing demand for lean meat implies that beef cattle improvement objectives should be broadened to include carcass traits (Mrode, 1988). As the North American beef cattle industry and improvement programs place increasing importance on carcass merit, methods of genetic evaluation that may increase accuracy of prediction for carcass traits should be investigated. Carcass trait evaluations may be improved by using information on genetically correlated early-life traits, such as preweaning growth, which are commonly measured and included in NCE.

Selection for preweaning growth traits is expected to result in a correlated response in traits such as carcass yield and its components (Koots et al., 1994b). In addition, genetic correlations between maternal components of preweaning traits and carcass traits are generally unavailable (Crews and Kemp, 1999).

Crews and Kemp (1999) conducted an analysis to estimate genetic parameters among preweaning growth and carcass yield traits. Additionally, they estimated the magnitude of maternal components of variance for standard carcass traits. Although the addition of maternal variance component terms did not improve models for carcass weight, non-zero maternal heritabilities were estimated for fat thickness, ribeye area and percent lean yield. For traits where maternal variances were greater than zero, maternal

heritability estimates were less than 0.09. When maternal variances appeared to be important, additive genetic variance estimates were reduced and residual variances increased, resulting in lower direct heritability. These results suggest that maternal effects may exist for carcass traits, but selection for these maternal effects may be antagonistic to direct selection for carcass yield and its components. Crews and Kemp (1999) further showed that carcass weight had positive genetic correlations with maternal components of birth (0.44) and weaning (0.64) weights.

Improvement in accuracy of carcass trait evaluations by adding preweaning growth information was measured as the decrease in prediction error variance of estimated breeding values resulting from more dense models. For carcass weight, ribeye area and percent lean yield, prediction error variance ratios were significantly decreased from their null expectation of one by adding birth and (or) weaning weight to the evaluation model. The largest prediction error variance decreases were noted for dams with fewer than three calves, although adding preweaning growth information did decrease carcass trait prediction error for most parents. Adding preweaning growth information was shown to improve carcass trait evaluations, and therefore may be recommended when information on carcass traits is limited (Crews and Kemp, 1999).

Ultrasound Prediction of Carcass Merit

Real time ultrasound (RTU) is considered a cost effective method for genetic evaluation of carcass merit in potential replacement beef cattle (Wilson, 1992). Real time ultrasound has been used to accurately predict carcass traits including subcutaneous fat thickness and longissimus muscle area in market animals prior to slaughter. Ultrasound technology has the potential to increase the numbers of animals with genetic values for carcass traits beyond those evaluated through time- and cost-intensive carcass progeny testing alone (Bertrand et al., 2000). Objectives of genetic improvement programs for carcass merit include the use of yearling- and (or) breeding-age RTU measures to select for component (e.g., fat and muscle) and composite (e.g., lean yield) measures of carcass merit and meat quality in slaughter progeny.

Yearling- and breeding-age RTU measurements on replacement bulls and heifers and their genetic correlations with carcass traits of slaughter progeny have been investigated in several recent studies (Moser et al., 1998; Devitt and Wilton, 2000; Reverter et al., 2000). Estimated genetic correlations between RTU measurements on breeding animals and corresponding carcass traits of slaughter animals have consistently been moderate to high and positive, but in most cases, less than unity (Moser et al., 1998; Devitt and Wilton, 2000; Reverter et al., 2000). To account for potential differences in underlying genetic control, evaluation models which treat RTU measures on breeding animals and carcass traits on slaughter animals as separate traits could be used. Further, extensive evidence that RTU measures on potential replacements of different genders at similar and dissimilar ages are genetically equivalent is lacking (Crews and Kemp, 2001a).

Real time ultrasound measures of longissimus muscle area and fat thickness were taken at yearling and 14 mo of age (breeding) on potential replacement composite bulls (n = 404) and heifers (n = 514). Carcass muscle area and fat thickness, hot carcass weight, estimated percent carcass lean yield, marbling score, Warner-Bratzler shear force, and 7-rib dissected seam fat and lean percentages were measured on steers (n = 235). Additive genetic variances for bull muscle area were 76 and 77% larger at yearling and breeding than corresponding heifer estimates, respectively. Heritability estimates were 0.61 and 0.52 for bull muscle area at yearling and breeding, and were 0.49 and 0.47 for heifer muscle area at yearling and breeding, respectively. Genetic correlations were 0.61 and 0.84 between bull and heifer muscle area

at yearling and breeding, respectively. Genetic correlations of steer carcass muscle area were 0.71 and 0.67 with yearling ultrasound muscle area in bulls and heifers, and were 0.73 and 0.79 with bull and heifer measures at breeding, respectively (Table 3). Heritability estimates for yearling bull and heifer ultrasound fat thickness were 0.50 and 0.44, while breeding-age estimates were 0.35 and 0.49, respectively. Bull and heifer ultrasound fat thickness had genetic correlations of 0.65 and 0.84 at yearling and breeding, respectively. Genetic correlations of steer carcass fat with yearling and breeding bull ultrasound fat were 0.23 and 0.21, while correlations with yearling and breeding heifer ultrasound fat were 0.66 and 0.86, respectively (Table 4). Live weight at yearling- and breeding-ages were genetically equivalent between bulls and heifers, and genetic correlations between live weights of potential replacements and steer carcass weight were greater than 0.80. Ultrasound measures of muscle area were also positively correlated with steer carcass and 7-rib percent lean yield, while ultrasound measures of fat were positively correlated with 7-rib seam fat percentage. Genetic correlations between ultrasound fat thickness and steer carcass marbling score were near zero. These results indicate that ultrasound muscle area and fat thickness may be under sufficiently different genetic control in bulls vs heifers to warrant their being considered separate traits for genetic evaluation. Further, ultrasound traits measured at yearling- and breeding-ages in potential replacements may be considered genetically different than corresponding carcass traits of steers.

Genetic Improvement of Carcass Merit Using Ultrasound

Genetic improvement programs for carcass merit require data from which breeding values can be estimated for potential replacement bulls and heifers. In addition to the drawbacks of cost and time, organized carcass progeny tests may also be limited by only providing accurate evaluations on a biased sample of a few popular sires, and little if any genetic information on females. The addition of live animal data, including RTU, to carcass evaluation programs provides the opportunity to not only evaluate a larger and more random sample of bulls within a population, but also to evaluate the genetic potential of females.

Carcass yield traits (e.g., hot carcass weight, longissimus muscle area, fat thickness) are generally considered to have moderate to high heritability (Koots et al., 1994a), and significant genetic correlation with growth and other live animal traits (Koots et al., 1994b; Crews and Kemp, 1999). Several recent studies have shown that RTU traits in bulls and heifers have positive and high genetic correlation with corresponding carcass traits in slaughter animals (Moser et al., 1998; Devitt and Wilton, 2000; Reverter et al., 2000), although genetic models which treat RTU and carcass traits as gender-specific (Crews and Kemp, 2001a) account for genetic correlations between genders and between live animal and carcass traits which are less than one. Selection for a combination of live animal and carcass traits would be expected to be effective. Several beef breed associations have carcass databases generated from field level performance programs and organized carcass progeny tests (Bertrand et al., 2000). Some have also begun collecting live RTU data to further facilitate genetic evaluation and improvement of carcass traits.

Live weight and ultrasound measures of fat thickness and longissimus muscle area were available on yearling bulls (n = 404) and heifers (n = 514), and carcass measures of weight, longissimus muscle area and fat thickness were available on steers (n = 235). Breeding values were estimated for carcass weight, muscle area, and fat thickness using only steer carcass data. Breeding values were also estimated for weight and ultrasound muscle area and fat thickness at yearling using data from bulls only then from both bulls and heifers where traits were considered gender specific. The combination of bull and heifer and steer data in a three trait model was also used to estimate breeding values. Generally, breeding values from the carcass data model were less accurate and distributed more closely around zero than

breeding values from the live data models, which could be at least partially explained by differences in phenotypic mean and heritability (Table 5). Mean accuracy of carcass trait breeding values ranged from 0.26 to 0.30 when estimated using only carcass data, and was increased by 37 to 150% as a result of adding live yearling data from bulls and heifers. The correlation of breeding values estimated using all available (live and carcass) data with breeding values estimated using either carcass or live data solely ranged from 0.28 to 0.98. Breeding values estimated with both live and carcass data had higher accuracy and were less closely distributed around zero than those estimate using only live or carcass data. Breeding values were more similar from all models for animals initially evaluated most accurately using only carcass data (Table 6). These results indicate that breeding values for carcass yield traits based solely on carcass vs live animal data were generally less accurate than breeding values estimated using live and carcass data in combination. Further, the addition of live animal data for genetic evaluation of carcass traits would be expected to result in significant increases in accuracy for young animals without progeny with carcass records (Crews and Kemp, 2001b).

Implications

Genetic evaluation using mature statistical procedures has become standard in the beef industry and now provides for prediction of genetic merit for many economically important beef production traits. Future developments in genetic evaluation will most likely be related to optimal economic combination of indicator traits into multiple trait selection programs. The implementation of these evaluation and selection tools within a planned breeding objective will be crucial. Recognized as the primary source of improved genetic resources, beef breed associations and similar alliances will play a critical role in transferring genetic merit to the commercial segment. Therefore, genetic evaluation systems should be developed considering the advantages and potential limitations of field data.

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Table 1. Summary statistics and genetic parameters for key components of the maternal productivity index (MPI) [a].

Item	Weaning weight, lb (WWTD)	Milk, lb (WWTM)	Cow weight, lb (COWWT)	Survival (SURV3)
n	3664	— [c]	3609	751
Mean	391	—	1094	64.71
σ^2_P [b]	2275	—	10889	0.15
		Genetic parameters [d]		
WWTD	0.19			
WWTM	-0.42	0.18		
COWWT	0.85	-0.17	0.50	
SURV3	-0.52	-0.01	-0.48	0.07

[a] Based on historical LRC Hereford data (adapted from Mwansa et al., 2001).
[b] Phenotypic (total) variance.
[c] Direct and maternal (milk) weaning weight are components of weaning weight.
[d] In the lower portion of the table, heritabilities are on the diagonal, genetic correlations below the diagonal.

Table 2. Genetic parameters [a] for components of the Terminal Sire Value (TSV).

	Birth weight (BWT)	Preweaning gain (WG)	Postweaning gain (PWG)	Lean yield (LY)	Marbling score (MAR)
units	kg	kg	kg	%	score
BWT	**0.42**	0.40	0.23	0.15	0.34
WG	0.26	**0.23**	0.39	-0.09	-0.11
PWG	0.16	0.20	**0.22**	-0.20	0.04
LY	0.10	0.25	-0.09	**0.38**	0.40
MAR	0.09	0.04	-0.03	0.18	**0.29**

[a] Heritabilities are on the diagonal in **bold**, genetic corelations above the diagonal, and phenotypic correlations below the diagonal (adapted from Caron and Kemp, 1999).

Table 3. Genetic parameter estimates [a] from longissimus muscle area models (from Crews and Kemp, 2001a).

	Bull		Heifer	
Trait [b]	Yearling (YBREA)	Breeding (BBREA)	Yearling (YHREA)	Breeding (BHREA)
Bull				
YBREA	**0.61 ± 0.16**	0.69	— [c]	—
BBREA	0.95 ± 0.02	**0.52 ± 0.14**	—	—
Heifer				
YHREA	0.61 ± 0.11	0.71 ± 0.09	**0.49 ± 0.12**	0.68
BHREA	0.70 ± 0.09	0.84 ± 0.06	0.95 ± 0.02	**0.47 ± 0.12**
Steer				
HCW	0.31 ± 0.21	0.37 ± 0.21	0.49 ± 0.18	0.34 ± 0.21
REA	0.71 ± 0.11	0.73 ± 0.10	0.67 ± 0.12	0.79 ± 0.08
FAT	0.16 ± 0.22	0.22 ± 0.22	-0.27 ± 0.20	-0.17 ± 0.22
PLY	0.35 ± 0.20	0.35 ± 0.21	0.51 ± 0.17	0.57 ± 0.15
MAR	0.31 ± 0.19	0.18 ± 0.21	-0.01 ± 0.21	0.11 ± 0.21
WBS	0.31 ± 0.22	0.24 ± 0.24	0.22 ± 0.23	0.05 ± 0.24
SMP	-0.66 ± 0.12	-0.68 ± 0.11	-0.12 ± 0.20	-0.40 ± 0.17
LNP	0.60 ± 0.14	0.60 ± 0.15	0.43 ± 0.18	0.60 ± 0.14

[a] Heritabilities (± SE) are on the diagonal in **bold**, while genetic correlations (± SE) are below the diagonal and residual correlations are above the diagonal.

[b] Bull real time ultrasound traits: YBREA = yearling bull longissimus muscle area, BBREA = breeding bull longissimus muscle area. Heifer real time ultrasound traits: YHREA = yearling heifer longissimus muscle area, BHREA = breeding heifer longissimus muscle area. Steer carcass traits: HCW = hot carcass weight (kg), REA = ribeye area (cm^2), FAT = 12^{th} rib fat thickness (mm), PLY = carcass lean yield (%), MAR = marbling score, WBS = Warner-Bratzler shear force (kg), SMP = 7-rib dissected seam fat (%), LNP = 7-rib dissected lean (%).

[c] Correlations not estimated.

Table 4. Genetic parameter estimates [a] from back fat models (from Crews and Kemp, 2001a).

	Bull		Heifer	
Trait [b]	Yearling (YBFAT)	Breeding (BBFAT)	Yearling (YHFAT)	Breeding (BHFAT)
Bull				
YBFAT	**0.50 ± 0.14**	0.45	—[c]	—
BBFAT	0.95 ± 0.02	**0.35 ± 0.12**	—	—
Heifer				
YHFAT	0.65 ± 0.11	0.44 ± 0.17	**0.44 ± 0.12**	0.47
BHFAT	0.61 ± 0.12	0.49 ± 0.16	0.93 ± 0.02	**0.49 ± 0.12**
Steer				
HCW	-0.27 ± 0.23	-0.33 ± 0.24	-0.10 ± 0.23	-0.08 ± 0.23
REA	-0.22 ± 0.21	-0.17 ± 0.24	-0.39 ± 0.18	-0.30 ± 0.19
FAT	0.23 ± 0.22	0.21 ± 0.24	0.66 ± 0.13	0.86 ± 0.06
PLY	-0.33 ± 0.21	-0.22 ± 0.25	-0.85 ± 0.06	-0.90 ± 0.04
MAR	-0.27 ± 0.21	-0.08 ± 0.24	-0.15 ± 0.21	-0.36 ± 0.18
WBS	0.03 ± 0.25	-0.05 ± 0.28	0.27 ± 0.23	-0.28 ± 0.22
SMP	0.18 ± 0.21	0.07 ± 0.23	0.75 ± 0.09	0.90 ± 0.04
LNP	-0.14 ± 0.23	-0.15 ± 0.25	-0.73 ± 0.11	-0.71 ± 0.11

[a] Heritabilities (± SE) are on the diagonal in **bold**, while genetic correlations (± SE) are below the diagonal and residual correlations are above the diagonal.

[b] Bull real time ultrasound traits: YBFAT = yearling bull fat thickness, BBFAT= breeding bull fat thickness. Heifer real time ultrasound traits: YHFAT = yearling heifer fat thickness, BHFAT = breeding heifer fat thickness. Steer carcass traits: HCW = hot carcass weight (kg), REA = ribeye area (cm^2), FAT = 12^{th} rib fat thickness (mm), PLY = carcass lean yield (%), MAR = marbling score, WBS = Warner-Bratzler shear force (kg), SMP = 7-rib dissected seam fat (%), LNP = 7-rib dissected lean (%).

[c] Correlations not estimated.

Table 5. Summary statistics [a] for breeding values and accuracies from live-only (L1, L2), carcass-only (C) and full (F) genetic models (from Crews and Kemp, 2001b).

	Breeding values				Accuracy	
Trait [b] - Model [c]	N	Mean	MIN	MAX	Mean	MAX
Weight						
YWT - L2 [d]	4095	-0.60	-101.1	93.1	0.78	0.95
HCW - C	4018	-0.28	-20.2	42.2	0.26	0.72
HCW - F	4095	-0.41	-49.1	44.5	0.65	0.84
Muscle area						
BREA - L1	4031	-0.40	-15.2	15.9	0.40	0.85
BREA - L2	4043	-0.49	-15.5	15.6	0.43	0.86
REA - C	4018	-0.06	-12.9	8.0	0.30	0.78
REA - F	4044	-0.38	-14.9	12.0	0.41	0.81
Fat thickness						
BFAT - L1	4031	0.02	-1.50	3.54	0.37	0.83
BFAT - L2	4043	0.02	-1.35	2.71	0.36	0.79
FAT - C	4018	0.11	-1.91	3.22	0.28	0.75
FAT - F	4044	0.12	-3.10	4.39	0.40	0.81

[a] N, MIN and MAX refer to numbers of animals with non-zero accuracy, minimum and maximum value, respectively.

[b] YWT = yearling weight, BREA = yearling bull RTU longissimus muscle area, BFAT = yearling bull RTU fat thickness. HCW, REA and FAT are steer hot carcass weight, longissimus muscle area and fat thickness.

[c] L1 = live animal (bull) data only, L2 = live animal (bull and heifer) data only, C = carcass data only, F = both live animal and carcass data included (i.e., L2 + C).

[d] Yearling weight assumed genetically equivalent between bulls and heifers.

Table 6. Summary statistics [a] for weight, longissimus muscle area and back fat thickness breeding values and accuracy changes by accuracy class [b] (from Crews and Kemp, 2001b).

Trait - Model [c]	Mean	MIN	MAX	Mean	MIN	MAX	Mean	MIN	MAX
	Low (N = 2637 [e])			Moderate (N = 1366)			High (N = 15)		
HCW-C BV	-0.1	-11.7	22.8	-0.7	-20.2	42.2	-2.0	-18.5	22.4
HCW-C acc	0.21	0.02	0.30	0.36	0.31	0.59	0.65	0.60	0.72
HCW-F BV	0.5	-39.5	44.5	-2.2	-49.1	38.5	-6.0	-26.2	27.1
HCW-F acc	0.63	0.14	0.77	0.69	0.51	0.82	0.83	0.82	0.84
Δ acc [d]	2.34	0.64	26.0	0.94	0.33	1.42	0.29	0.17	0.36
	Low (N = 1994)			Moderate (N = 1987)			High (N = 35)		
REA-C BV	0.0	-4.9	4.8	-0.2	-9.9	7.9	0.4	-12.9	8.0
REA-C acc	0.21	0.02	0.30	0.38	0.31	0.59	0.66	0.60	0.78
REA-F BV	-0.1	-11.9	10.8	-0.7	-13.7	12.0	-0.2	-14.9	8.7
REA-F acc	0.31	0.04	0.60	0.52	0.34	0.74	0.75	0.63	0.81
Δ acc [d]	0.46	0.08	4.22	0.39	0.03	0.97	0.14	0.03	0.23
	Low (N = 2269)			Moderate (N = 1719)			High (N = 24)		
FAT-C BV	0.03	-1.11	1.54	0.23	-1.91	3.22	0.28	-1.28	1.99
FAT-C acc	0.21	0.02	0.30	0.36	0.31	0.59	0.66	0.60	0.75
FAT-F BV	0.01	-2.71	4.39	0.26	-3.10	4.10	0.30	-1.65	2.53
FAT-F acc	0.31	0.02	0.66	0.51	0.35	0.77	0.76	0.68	0.81
Δ acc [d]	0.51	0.04	5.67	0.39	0.04	1.13	0.17	0.04	0.30

[a] MIN and MAX are minimum and maximum values.

[b] Classes designated on the basis of carcass-only (C) model breeding value accuracy (acc), where accuracy ≤ 0.30 = Low, $0.30 <$ accuracy < 0.60 = Moderate, and accuracy ≥ 0.60 = High.

[c] HCW, REA and FAT are steer hot carcass weight, longissimus muscle area and fat thickness, respectively. C and F refer to carcass-only and full genetic models, respectively. BV and acc are breeding value and accuracy, respectively.

[d] Change (increase) in breeding value accuracy from model C to model F (i.e., due to addition of live animal data to carcass data), Δ acc = (REA-F acc — REA-C acc) / (REA-C acc)

[e] Numbers varied by trait, model and accuracy class due to numbers of observations and number of non-zero accuracy values.

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The Bovine Genome Map and Molecular Approaches to Genetic Improvement

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Key Points

- The bovine genome has become the best understood genome among livestock species.
- The pace of discovery is increasing with initiatives in the private and public sectors.
- Although many Quantitative Trait Loci (QTL) have been discovered underlying production and quality traits, the genes themselves are largely unknown.
- The relevance of many QTL discovered in research herds has yet to be determined in commercial herds.
- New approaches to gene discovery enable us, in some cases, to move directly into commercial populations, the limiting factor remains the ability to accurately measure phenotype.
- The application of Marker Assisted Selection (MAS) and other genomics based technologies in animal improvement, will depend on our ability to measure multiple genotypes and phenotypes. In other words a synthesis of more traditional quantiative and new molecular genetics based approaches.

Introduction

Over the last 15 years unimagined progress has been made in the understanding of the structure of the mammalian, and many other genomes. A watershed was reached earlier this year with the announcement of the draft human genome sequence. This heralded not the end but the beginning of genomics applied across a plethora of species. Among the livestock species, the cow is the best defined in terms of genome structure. Medium density physical and linkage maps are available and more importantly, the comparative map between cow and man is improving to the point the direct comparisons of genome structure and function are now possible. Genome mapping efforts in the cow have largely been focused on the discovery of genes or multiple loci, termed Quantitative Trait Loci, underlying economically important traits such as those important in production efficiency or product quality. These efforts have largely been successful, however the genes underlying these QTL remain largely unknown and thus the technology has been difficult to apply to extensive industries such as beef. The frequency and effect of many of the genes and QTL remain to be

determined in commercial herds before a thorough economic analysis of their value can be undertaken. The interaction of QTL with one another and their effect on traits other than the ones described also has implications as to their utility.

New methodologies are becoming available that will aid significantly in the analysis of the bovine genome and allow work to be done directly in commercial herds rather than relying entirely on specially generated pedigrees designed to maximize the genetic variation. Methods based on Identity by Descent (IBD) of chromosomal regions within particular cattle populations greatly increase our ability to detect genes affecting economically important traits. The limiting factor remains our ability to accurately measure phenotypic variation. For many traits that are difficult or expensive to measure in the commercial environment, research herds will remain the only way forward.

Finally, the last year has seen the first commercial outcomes of the QTL mapping studies undertaken in cattle over the past decade. Genetic variation that significantly influences traits such as marbling can now be measured and the test is being provided commercially. The full potential however will not be realised until tests for multiple traits are available and a better understanding of QTL interaction and pleiotrophic effects is achieved. Gene tests will also need to be applied in the context of more traditional selection schemes to avoid negative affects that may result from selection on markers alone.

The Bovine Genome Project

The gene and genetic maps of the cow are the most advanced among the livestock species. Medium density genetic maps were published in 1997 (Barendse et al., 1997; Kappes et al., 1997). These maps comprise over 3000 genes or markers allowing moderately high precision in gene association studies based on interval mapping or IBD approaches. A wealth of information is available to researchers through web-based informatics (http://spinal.tag.csiro.au/cgd.html, http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl, http://bos.cvm.tamu.edu/bovgbase.html, http://sol.marc.usda.gov/). More recently, physical gene maps have approached the resolution of the linkage maps. Methods based on Whole Genome Radiation Mapping have placed over 1000 genes and markers on this physical map (Band et al., 2000; http://bos.cvm.tamu.edu/htmls/rhbta.html) and more importantly greatly improved our understanding of the human vs. cow comparative map.

An even more ambitious effort to fine map the bovine genome has commenced. Our group at the University of Alberta and the AAFC group led by Dr. Bernhard Benkel is collaborating with scientists at the Genome Sequencing Centre, Vancouver, the USDA and other international groups in the USA and Europe to produce the finest possible resolution gene map of the cow (http://www.bcgsc.bc.ca/projects/bovine_mapping/). This map is in fact the penultimate step towards sequencing the bovine genome, an undertaking no one would have contemplated as possible as little as 5 years ago.

Applications

So what will all this mean to the way we select and manage the animals used to produce beef? In short our ability to find and characterise genes, which may be important for production or quality is and will be considerably enhanced by these basic scientific advances. To date most gene discovery programs aimed at beef cattle have been undertaken in research herds specifically generated to maximise the observed genetic variation in the animals (Davis et al., 1998; Hetzel et al., 1997; Keele et al., 1998; Stone et al., 1999). This has been done for good reason. The approaches taken rely on the animals to be heterozygous both at the QTL and the marker loci. Animals that are

homozygous, even for a desirable allele, are not detectable using the interval mapping approaches. The approach also means that genes or gene markers identified in these atypical research populations must be tested to determine if the causal alleles are segregating in commercial animals and to determine the effect of variation of the genetic background on the genes.

The increased density of the cattle linkage map has meant that approaches based on Identity by Decent of shared haplotypes can now be applied to cattle populations (Fanir et al., 2001; Riquet et al., 1999). Essentially this approach looks at the inheritance of ancestral chromosomal segments in populations that are derived from a limited number of founders. Common haplotypes can be associated with variations in phenotype regardless of whether the animal is homozygous or heterozygous for the chromosomal segment being examined. The one criterion is that the animals are interrelated enough that the associations are maintained in the population. Fortunately this is the case for many cattle populations such as commercial breeding lines.

A number of growth QTL have been reported by various groups (Davis et al., 1998; Elo et al., 1999; Stone et al., 1999; Casas et al., 2000). We have used an IBD approach to map genes associated with growth in breeding lines of beef cattle. We tested for these QTL in two breeding lines from Beefbooster Inc. Figure 1 shows the analysis of bovine chromosome 5 in the Beefbooster M1 line. The presence of growth QTL on this chromosome were confirmed by this study. Indeed three regions of the chromosome were identified at a resolution far beyond that achieved in the other studies using interval mapping approaches. One region was not developmentally specific, affecting growth at all ages. One region affected only pre-weaning average daily gain while the third affected only average daily gain. The results were further confirmed by analysis of the Beefbooster M3 line. This line was derived independently from the M1 line, so an association in the same chromosomal region is very strong evidence that the result is true. As can be seen in Figure 2 the same regions were associated with growth traits in the M3 line. Interestingly there was some variation in the developmental stages at which these QTL were acting. This may be due to the different genetic background of the M3 animals or the presence of different alleles of the genes in the different populations. As the density of the genetic maps improves, and with the reduction in genotyping costs expected as new technologies such as Single Nucleotide Polymorphism (SNP) analysis are developed, we will be able to extend this approach beyond breeding lines to more unrelated animals such as within breed.

With the development of resources such as large Expressed Sequence Tags (EST) databases and accompanying cDNA arrays new approaches are now available to study the tissue specific expression of genes on a large scale (Eisen et al., 1998; Greenfield, 2000). Combined EST mapping with QTL mapping approaches will enable the more rapid identification of candidate genes that might underlie QTL. These resources will also allow the identification of genes important in specific metabolic pathways affecting production or quality traits. Essentially these EST databanks are compiled by sequencing large numbers of genes expressed in particular tissues. Comparative bioinformatics approaches can identify many of these genes and assign function to them. A large set of cattle expressed sequence tags is being assembled at the University of Alberta. The source of the genes has been muscle, adipose and various tissues from the bovine gastrointestinal tract. DNA from these genes can be arrayed on nylon filters or glass slides and these arrays used to measure the levels of expression of the genes in various tissues and under various treatments. Figure 3 shows a filter array of some 800 genes derived from muscle and adipose tissues. The number of genes represented can reach many times this if glass arrays are used, permitting the simultaneous analysis of the significant proportion of the expressed genome in one experiment. We are using these arrays to determine the genes important in determining the sites and amounts of adipose tissues in animals of various genotypes under various environments, as well as to better understand the processes in the GI tract which may be associated with nutrient utilisation. To date over 5000 sequences have been generated from muscle, adipose, duodenum, rumen, omasum and abomasum.

Given all this new technology is there still a need for research herds? The answer of course is yes. Our ability to link genes to specific functions related to production and quality is limited by our ability to measure the traits in animal populations. The very nature of the technology means that it is most valuable for traits that are difficult or expensive to measure. The environment under which the animals are grown and the traits measured have to be controlled, sometimes to a degree that would make working with commercial herds impossible. Examples of such traits are some meat quality traits and net feed efficiency.

Implications

We are now seeing the first gene tests commercialised for some production and quality traits. However, as yet most QTL identified have been characterised only in terms of flanking DNA markers. This fact limits their application to large breeding lines in enterprises that are vertically integrated and can capture the value of the marker tests. It is expected over the coming years that more and more of the genes underlying QTL will be identified. The temptation will be to select heavily on these gene tests, in order to quickly realize some value from the tests. However, caution is warranted as too much weight on any one trait may have negative effects on the overall genetic merit. Marker Assisted Selection, particularly when only a few markers are considered will have to be applied in the context of phenotypic based selection schemes. Our understanding of gene interactions and pleitrophic effects of genes underlying production and quality traits will need to become more sophisticated in order for true long term gain to be achieved.

Acknowledgments

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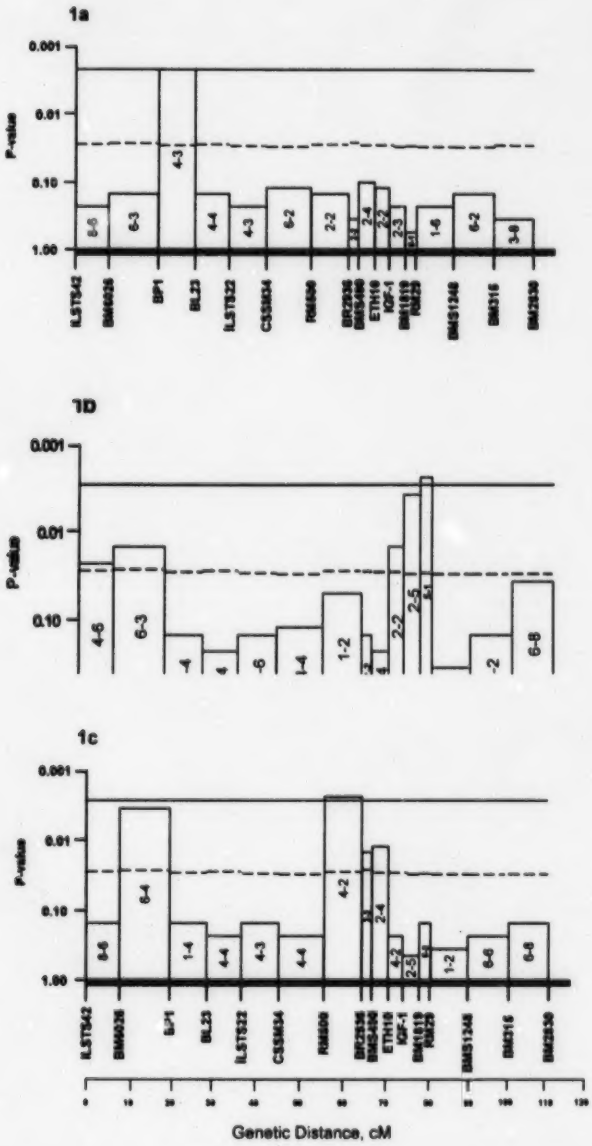

Figure 1. Haplotypes with the lowest p-values between two adjacent loci along BTA5 for birth weight (1a), pre-weaning average daily gain (1b) and average daily gain on feed (1c) in M1 line. The map genetic distances of the haplotypes were indicated as in (1c). The dashed line ----- represents the comparison-wise p-value threshold level. The solid line — represents the chromosome-wise p-value threshold level.

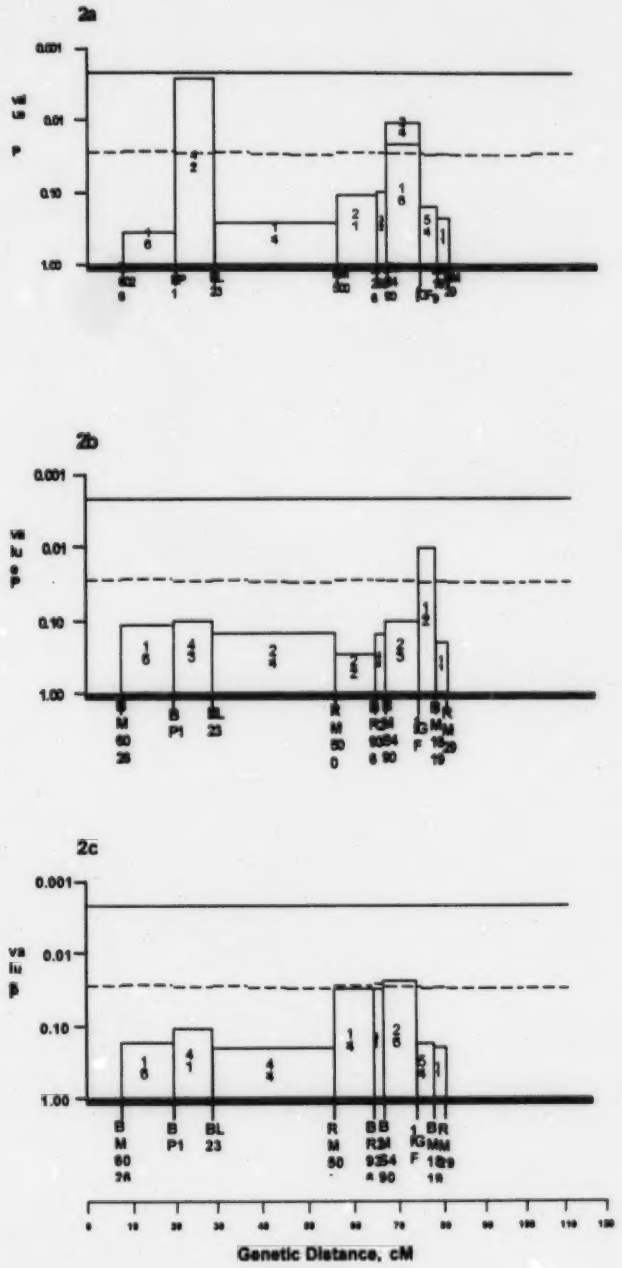

Figure 2. Haplotypes with the lowest p-values between two adjacent loci along BTA5 for birth weight (a) pre-weaning average daily gain (b) and average daily gain on feed (c) in M3 line. The map genetic distances of the haplotypes were indicated as in (c). The dashed line ----- represents the comparison-wise p-value threshold level. The solid line — represents the chromosome-wise p-value threshold level.

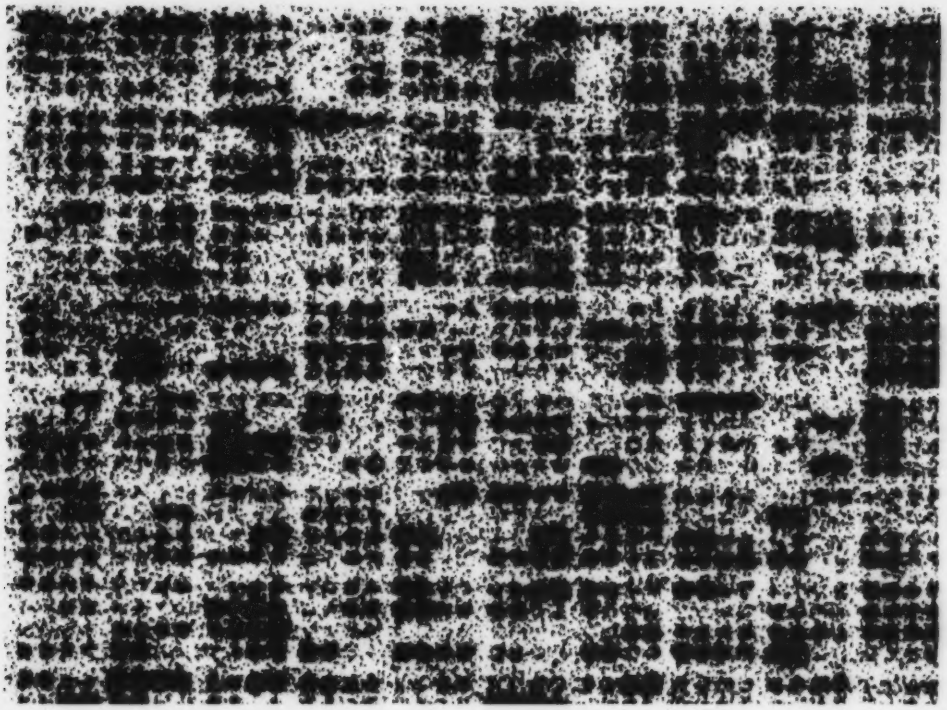

Figure 3. A filter array of sequences derived from muscle and adipose tissue. This filter contains 768 individual sequences isolated at the University of Alberta. CDNA was spotted onto the filter using a 96 pin spotting tool on a Beckman BioMek 2000 robot. The filter has been probes with 33P labeled cDNA from mouse 3T3 preadipocyte cells.

Expected Progeny Differences and Molecular Information for Genetic Improvement of Economic Merit

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Key Points

- More information on genetics of beef cattle is becoming available through both new and better Expected Progeny Differences (EPD) on more traits and through molecular information.
- More rapid genetic change can be made by using both EPD and molecular information.
- Choices of breeds, crosses and animals depend on economics at the commercial level.
- New tools are available to help combine economic considerations, including market preferences, with genetic information to optimize the genetics for each production situation.

Introduction

Both new molecular information and improved data recording (for better expected progeny differences [EPD] for more traits) offer new opportunities for improved genetics in the beef industry. The need to match genetics with management to economically produce animals under emerging price grid systems is becoming an important issue. New computing tools are being developed to help producers deal effectively with these new opportunities and issues. The purpose of this presentation is to provide information on definitions of economic merit, ways of increasing rates of genetic improvement through a combination of EPD and molecular information, and approaches for combining information on a variety of traits into easily-used guides.

Genetic Improvement of Economic Merit

Genetic improvement takes place at two key levels in the beef industry. One is the level of seedstock development, whether purebred or composite. The other is the level of commercial production, where genetics is one of the interacting components of production. At the commercial level, improving the genetic component of production, while considering the combination of genetics with nutrition and other management factors, can be the result of selecting the best animals and making use of crossbreeding, such as by using a terminal crossbreeding program. Traditionally, crossbreeding has been of major interest to cow-calf producers because of the heterosis effect resulting from combining the genes of different populations or breeds and the boost in productivity from using sires with more

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growth potential than in the cow breeds (within the limits of avoiding calving problems). Recently, much interest has been developing in the area of "specification" feeders for the feedlot, having the genetics to match the feed supplies and market demands.

At the seedstock level, the challenge is to produce a genetically-superior animal which will fit with commercial demands. The challenge includes identifying the role of the breed or composite in the production program, as well as identifying which traits need to be improved and by how much.

Defining Economic Merit

Economic merit depends on the prices received for the product and the costs of production. It is important that these prices and costs be measured at the commercial progeny level. It is also important that these prices and costs be applicable when the progeny are in production. Usually, long-term averages of prices and costs are used because they are the best prediction of what will be happening at the time progeny are performing. For seedstock producers, the time lines are even larger than for commercial producers since their challenge is to develop lines of cattle for use over several years in the future.

Using Expected Progeny Differences and Molecular Information

Genetic improvement depends on changes in the frequencies of genes in the population, either by selecting animals using EPD or molecular data or both (Figure 1). There are some traits affected only by a single gene, such as the gene for polled or horned. Even if the location of such a gene on the chromosome is not known, breeding records can tell us what the genotype of the animal is (although identification of carriers can be made much easier if specific genetic tests exist). Selection can then quickly change the genetics of the population for that trait. In many cases though, there are many, many unknown genes that affect a trait. Then, we have a wide range of levels of traits, such as the continuous nature of growth rate. We then have the situation where we have many unknown genes, but we do have observed records (known as phenotypes). If we have the records and know the pedigrees (family structures), we can estimate the value of the genes (known as Expected Progeny Differences or EPD). We can, of course, only obtain EPD for traits that we measure. In some cases, we rely on measurements of indicator traits to bring about the changes we want in a trait. An example of an indicator trait is scrotal circumference which is an indicator of fertility. The important final step is selection, which results in some animals being kept as parents and others not, resulting in a change in the frequency of the "good" genes.

Molecular data is used in several ways. One is for cases in which genes are identified and for which a genetic test is available. To date, there are only a few such genes identified , mostly in the area of eliminating deleterious recessive genes. Animals can be tested as to whether or not they are carriers of the gene and, if so, they can be deleted as prospective parents. The frequency of desirable genes could be increased in a similar way, for example, by using animals with the myostatin gene.

An approach called Marker Assisted Introgression (MAI) is another use of molecular information. In some cases, there are genetic markers which are close to the gene of interest, even though the gene's location is not yet known exactly. Animals with the marker could be selected to increase the frequency of the desired gene. An example in pigs is the use of a marker for the estrogen receptor gene which is associated with litter size.

Another approach is Marker Assisted Selection (MAS). This approach is used for cases in which markers have been found which are associated with a specific trait. For example, several markers have been found for twinning rate. Two of these markers are being incorporated into a selection program at the Meat Animal Research Centre in Nebraska aimed at developing a twinning population. Considerable theoretical work is being carried out on techniques for combining markers with records on traits to obtain EPD. Future progress in applying these techniques depends on identification of more markers and even more so on having records on traits. This is especially true for traits that are expensive to measure, such as tenderness, feed efficiency and fertility.

Putting It All Together

Putting it all together starts with money. The beef industry involves many segments, from cow-calf to feedlot to packer to retailer. In total, there are revenues and expenses that influence production decisions. Beef quality traits are important to the extent they affect revenues. In fact, all traits are important simply to the extent to which they affect either revenues or expenses.

As an example of the connection between economics and genetic improvement, recent work at Lethbridge has led to a suggested maternal productivity index for the Hereford breed. The target of the breeders is to develop cattle that will be superior for maternal traits. Economic considerations were limited in this example to commercial cow-calf production. Revenue was identified as coming from the weaned calf, while costs were identified as those for feeding and replacing cows in the herd. The economic value of weaning weight (as a function of the calf's genetic ability to grow) was then based on sale prices of calves. Economic values were also found for cow weight (a trait that can be measured and that is closely associated with feed intake), maternal contribution to weaning weight (determined as the extra weight of calf from a higher milk-producing cow less the extra feed required for the extra milk) and survival rate (a trait measured as the probability of surviving to produce at least a third calf which has an economic value based on the cost of replacement heifers less cow salvage costs). Note that in this approach, calf weights, cow weights, whole herd recording of cows producing calves each year and pedigree information are all needed. Genetic evaluations (EPD) for each trait can then be combined into one index to use in selecting animals, by weighting the EPD for each trait by its economic value. Preliminary results to date have led to an index as follows:

Maternal Economic Index = \$3.00 x EPD for Weaning Weight Direct (kg)
+ \$2.80 x EPD for Weaning Weight Maternal (kg)
- \$0.48 x EPD for Cow Weight (kg)
+ \$361.00 x EPD for Cow Survival (%)

A simulation study was conducted to show the expected change in these traits for a case where there were measurements on all four traits, and selection of sires was based on information on 20 calves with weaning weight and 10 daughters going on to have their mature weights recorded and possibilities for having calved for a third time. The expected changes in a population under this type of selection with a moderate level of selection applied were found to be:

3 kg extra weaning weight/calf for extra growth,
3 kg extra weaning weight/calf for extra milk,
5 kg increase in cow weight, and
3% increase in survival rate.

It is interesting to note that even though increased cow weight leads to more costs because of increased feed requirements, an increase in cow weight is expected because of the association of cow weight with calf growth. However, the proportionate increase in cow weight expected is not nearly as large as that for calf weight. In other words, the change in calf weight was a higher percentage of average calf weight than the parallel percentage for cow weight.

Another very new approach to include economics in selection decisions is the development of internet-based selection tools (Wilton et al., 1998; Lazenby et al., 1998). The purpose of these tools is to enable producers to choose sires that will contribute the most to the profitability of their operations. The example presented here is the choice of sires under two different pricing grids. In this example, revenues are considered to be influenced by market weights (including discounts for under- or over-weight carcasses), retail yield and marbling. Costs are considered to be those at both the cow-calf and feedlot levels. Feed is the major expense and feed intake measurements are assured to be available for prospective sires in their post-weaning phase of growth. Typical natural service offspring numbers were assumed for both market offspring and cows in the herd (these can be changed in customized applications). Typical feeding programs and use in crossbreeding programs are used in base calculations, with these also being subject to customization. Progeny performance levels for cow traits, feedlot traits and carcass traits are predicted from EPD on sires. For example, steer progeny performance in terms of retail yield is predicted from EPD for growth rate, backfat and rib-eye area. Steer progeny performance for proportions of progeny in each marbling class is predicted from EPD for marbling. Fertility levels are predicted from EPD for scrotal circumference currently, but could be more precisely predicted from EPD for survival rates. As more molecular information is available, it can be incorporated into refining these predictions. The end result of the use of genetic evaluations in a calculation of net economic value in terms of dollars and cents for each sire for each situation.

For example, we have calculated the net economics for two bulls under both a standard marketing situation and under a price grid that pays more for marbling. Under the standard pricing, the bulls had values of $3640 and $1429, respectively, which represent the values of using the bulls in a herd compared to an average bull. Under the premium grid pricing system, the two bulls had values of $640 and $4330, respectively. The second bull had a better evaluation in the premium situation because he actually had a higher EPD for marbling and a higher proportion of progeny grading AAA or better. Clearly, the best bull to use depends on the production and marketing situation. Seedstock producers can use the results applicable to typical situations for their bull buyers in order to develop their own breeding programs. In total, the internet approach makes it possible for users to rank sires for profitability for their own situations.

Implications

Genetic evaluations can be made more accurate by the combined use of EPD and molecular information. These genetic evaluations can be combined with economic information to provide producers with rankings of animals for total economic worth. Innovative new approaches to allow customization of rankings for particular situations are now available. Research in the near future will make more accurate evaluations possible along with more flexibility in computation of total economic values.

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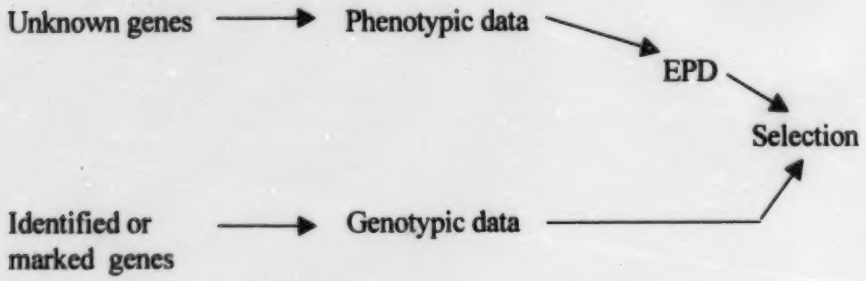

Figure 1. Molecular data and Expected Progeny Differences (EPD) in genetic improvement.

Community Genomics – The Key to the Rumen?

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Key Points

- Although the rumen is often described as the most intensively studied microbial ecosystem, recent studies show that less than 15% of the bacteria present in the rumen have been cultured and characterized.

- New techniques are available that allow the complete genetic resources of a microbial community (the "metagenome") to be defined and made available for analysis without the need to cultivate the community members.

- The resulting understanding of the real membership and functions of the rumen microbial community will allow rational approaches to issues such as productivity and product quality, methane production, phosphorous pollution, development of antibiotic resistant microbial populations, and the transmission of pathogens such as Escherichia coli O157:H7 between animals and humans.

Introduction

Decades of attempts to culture a significant part of the rumen microbial community have failed. Although the rumen is often described as the most intensively studied microbial ecosystem, recent estimates based on sequencing of randomly cloned 16S rDNA sequences indicate that less than 15% of the bacteria present in the rumen are closely related to bacteria we have been able culture and characterize. The rumen microbial community is now estimated to include from 500 to >1,000 native species, including bacteria, protozoa and fungi. Even numerically dominant groups of bacteria identified by cloning of 16S rRNA genes from DNA isolated from rumen samples have never been identified among cultivated organisms. As a consequence, rational approaches to the manipulation of the rumen microbial community to improve rumen function have not been possible. Substantial improvements in the efficiency of ruminant production have been achieved through empirical evaluation of rumen function and animal performance, but in the absence of an adequate understanding of the composition and functions of the rumen microbial community this approach is slow, expensive, and often unsuccessful.

While we estimate that only 15% of the dominant species in the rumen have been cultured, the situation in other natural environments such as soils is even worse, with recent estimates suggesting that less that 0.1% of the microbial species present have been cultured (Relman and Strauss, 1999). The reasons for our inability to cultivate a large proportion of the bacteria that live in the rumen in natural environments are still not clear. Contributing factors probably include lack of recognition (i.e., many more may very well

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have been cultured but were never sufficiently well characterized to be recognized as unique), failure to provide appropriate conditions (bacteria live in very localized micro-environments, and media that approximate the overall environment may not meet their specific needs), and dependence by bacteria on a consortium of other species to provide appropriate nutrients or environmental conditions. Whatever the reasons, it is now generally accepted that culture-based approaches are unlikely in themselves to provide an accurate model of microbial community function.

However, large scale sequencing projects such as the human genome project have resulted in the development of new techniques and approaches for genome mapping that promise to revolutionize the analysis of complex microbial communities. These approaches allow the complete genetic resources of the community (the "metagenome") to be defined and made available for analysis without the need to cultivate the community members. The key development was the ability to clone and maintain large segments of DNA (100-300 kilobases (kb)) in Escherichia coli using bacterial artificial chromosome (BAC) vectors (Shizuya et al., 1992). Since the average size of a microbial genome is about 3,000 kb, a complete microbial genome can be represented by a set of 30-50 clones. If DNA is isolated from a microbial community and cloned in BAC vectors, overlapping clones can be identified and sets of clones (contigs) selected that correspond to individual chromosomes (Soderlund et al., 1997; Hong, 1997; Zhang and Wing, 1997).

Rondon et al. (2000) recently demonstrated the preparation of a soil microbial community metagenome library (25,000 45-kb clones, or as many as 106 genes), and were able to use the library for both community analysis (using 16S rDNA as a marker) and the identification of clones expressing interesting genes (hydrolytic enzymes and antimicrobial peptides). Similarly, Beja et al. (2000) demonstrated the utility of a similar approach in the analysis of a marine microbial community.

In determining the size of clone library that would have to be prepared to be reasonably sure of getting the complete set of genomes of the rumen community members, the quality of the DNA preparation is critically important. If insert size averages 100 kb, a library of 50,000 clones is unlikely to cover the complete genome of any organism that comprises <0.5% of the community (see Figure 1 below). If the average insert size is increased to 200 kb, even organisms that comprise only 0.2% of the community are likely to be fully represented, and most (>90%) of the genes of less abundant (0.1% of the community) organisms would be represented. There are some concerns that the presence of closely related organisms in the rumen could make contig assembly difficult. However, the recent development of effective approaches to contig assembly in BAC libraries from polyploid organisms (Lin et al., 2000) suggests that this will not present a serious barrier to contig assembly.

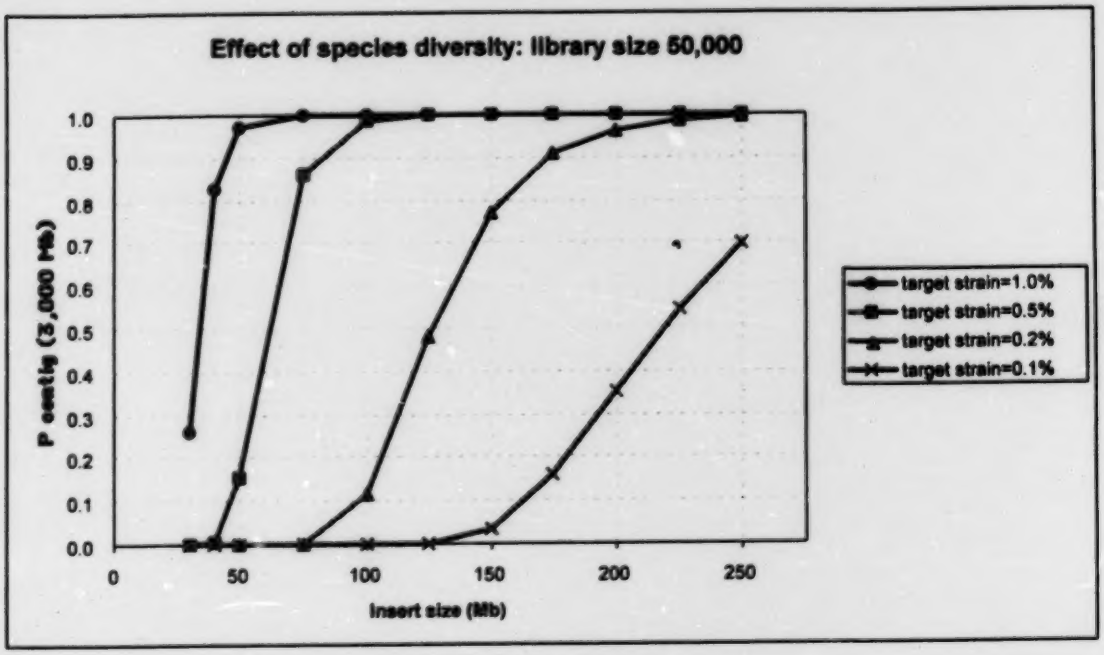

Figure 1. Probability of assembling the complete genome of a member of a microbial community. Vertical axis: probability of recovering the complete genome; horizontal axis: average size of the DNA fragments isolated from the community.

Up to the present time, the technique of choice for isolating environmental DNA has been based on physical breakage of bacterial cells by shaking at high speed in the presence of small glass beads ("bead beating"). Present bead beating methods allow isolation of representative DNA but limit fragment sizes to about 50 kb, which would result in the need for impossibly large libraries (many millions of clones) to achieve a reasonable probability of genome assembly. We have now developed cryogenic grinding methods that allow the isolation of DNA with a molecular weight >200 kb from rumen samples (Figure 2).

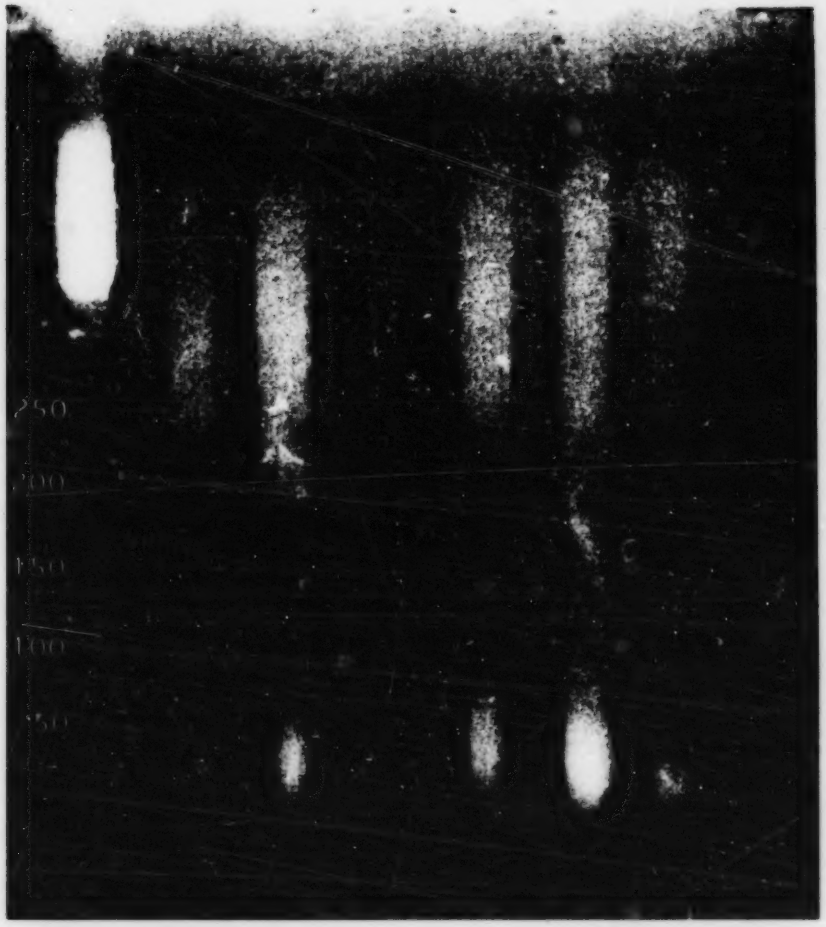

Figure 2. Cryogenic Grinding Preparation of Rumen Microbial DNA. Numbers indicate the size of the DNA fragments in kb.

These technical developments, together with the associated development of robotic systems for colony recognition and picking, library replication, BAC DNA preparation, and sequence analysis, make the analysis of complex microbial communities through the construction and analysis of community DNA libraries feasible.

The successful application of this technology will allow us to define and provide ordered access to the collective rumen community genome (metagenome). This will provide us with ban accurate picture of not just what organisms are present, but also what the organisms do, without ever isolating and cultivating the organisms. The defined contigs representing the complete genomes of the predominant organisms in the community will provide easy access to the sequence information needed for the design of arrays for rapid analysis of the effect of nutritional status, stress, and disease on the rumen microbial

community structure and function. They will also provide an ordered, uninterrupted source of materials for efficient genome sequencing of dominant organisms.

Implications

Full knowledge of the capabilities and phylogeny of the members of the communities will allow focussed studies of microbial interactions with other microorganisms and with the host animal, and effective manipulation of community structure. Basic understanding of the microbial ecology of the rumen will allow rational approaches to issues such as methane production, phosphorous pollution, development of antibiotic resistant microbial populations, and the transmission of pathogens such as Escherichia coli O157:H7 between animals and humans. This will support the development of new tools to promote animal health, product quality, and the productivity and sustainability of the livestock industries.

The community genome library will provide other important benefits including immediate access to the complete hydrolytic enzyme complement, and in most cases complete biochemical pathways, of the rumen, and to other bioactive peptides, including pharmaceuticals and enzymes involved in intermediary metabolism, that have potential applications in agriculture, industry and medicine.

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Session VIII - Reproduction

Speakers:

Dr. George Seidel
Dr. Sylvie Bilodeau-Goeseels
Dr. John Kastelic

Procedures for Sexing Bovine Sperm[a]

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Key Points

- Sperm can be sexed with 90% accuracy.
- The current speed of sexing is about 5,000 live sperm of each sex per second, remarkably fast considering that each sperm is individually sexed.
- Although fast, sperm sexing is not fast enough to use standard numbers of sperm per artificial insemination dose.
- With well managed heifers, pregnancy rates with low doses of sexed sperm are 70-80% of those with unsexed sperm with normal sperm numbers. Pregnancy rates are lower in cows.
- Calves from sexed sperm appear to be normal.
- Sexed, frozen semen from a few bulls may be available commercially in 2002, probably at a premium of US$ 30-50 per straw.

Introduction

Many methods of sexing sperm have been attempted, and there have been sufficient failures to cause much skepticism about sperm-sexing claims. However, recent work has clearly shown that one method is successful with various species, including cattle and horses. This procedure, flow cytometry/cell sorting, is 85 to 95% accurate. It was developed for sexing live sperm by Dr. Larry Johnson in the United States Department of Agriculture laboratory at Beltsville, Maryland, and patented by the U.S. government. Exclusive rights to the patent have been assigned to XY, Inc., a company in Colorado, for applications in non-human mammals. XY, Inc. has subsequently spent millions of dollars in further research, including development of faster sperm sorters. Much of this work was done in collaboration with Colorado State University.

Procedures for Sexing Sperm

Bovine X-sperm, which produce heifers, have 3.8% more DNA (genetic material) than Y-sperm, which produce bulls. Freshly collected sperm are bathed in a DNA-binding stain for about 45 min. This particular stain fluoresces a deep blue when exposed to short wavelength light, provided by a laser. Thus, X-sperm give off 3.8% more blue light under these conditions than Y-sperm. Although a 3.8% difference is too small for people to discriminate using a simple microscope, with the proper electronic equipment plus a powerful computer, this difference can be detected rapidly and accurately for most, but not all sperm.

For the actual sexing procedure, fluid containing the stained sperm passes a detector that measures the brightness of the sperm-bound dye. The fluid then exits a vibrating nozzle that causes individual droplets to form. Ideally, each droplet would contain a single sperm; in practice, over half of the droplets contain no sperm, 20-30% of droplets contain one sperm, and a few droplets contain 2 or more sperm.

Information from the detector is processed by the computer and relayed to the nozzle where a positive charge is given to droplets containing X-sperm, a negative charge, to droplets with Y-sperm, and no charge to droplets with 1 X- and 1 Y-sperm, no sperm, unsexable sperm, or dead sperm. The exiting droplets pass by positively and negatively charged plates; since opposite charges attract, the positive droplets veer toward the negative plate; the negatively charged droplets, toward the positive plate, and the uncharged droplets are unaffected and simply continue falling. These three streams are collected separately, resulting in physical separation of sperm by sex.

Speed of Sexing

This sexing procedure has been improved greatly over the past several years, and improvements continue to be made. About 90,000 droplets are formed per second, and they exit the nozzle at nearly 100 km per hour. Currently about 5,000 live sperm of each sex at about 90% accuracy can routinely be collected per second (nearly double this under ideal circumstances), the rate depending on characteristics of individual semen samples. As procedures improve, these rates likely will increase considerably. Possibly multi-nozzle sorters will be developed, analogous to multi-cylinder engines.

Numbers of Sexed Sperm Needed for Successful Artificial Insemination

Performance of current sperm sorters is impressive; routinely, more than 15,000,000 sperm of each sex can be sorted per hour. However, this is just in the range of the number of sperm in a typical dose of semen for artificial insemination. Thus, even a 2- or 3-fold increase in sorting throughput would be too slow for standard AI practices. It would, however, be quite practical for in vitro fertilization.

For several years, we have been breeding heifers successfully with sexed sperm. Insemination of well managed heifers with fewer than 1 million unsexed frozen-thawed sperm per dose results in near normal pregnancy rates with semen of some bulls if skilled inseminators are employed. Similarly, Dutch workers recently found that non-return rates were normal when breeding dairy cows with fewer than 2,000,000 sperm with semen from the most fertile 20% of bulls (Den Daas et al., 1998).

Several years ago, we used the concept of low-dose insemination to inseminate 1,000 heifers with sexed sperm along with 370 heifers with unsexed sperm from the same bulls (Seidel et al., 1999). Sperm were from 22 bulls selected for good semen quality; actual fertility information was not available for most of these bulls before the trials. The 2-month pregnancy rates determined by ultrasound averaged 47% for sexed sperm, mostly at 1 to 3 million frozen sperm/dose, and 60% for unsexed, control sperm, mostly at 20,000,000 frozen-thawed sperm/dose. We and others have since confirmed these results with thousands of additional heifers. However, pregnancy rates are lower when heifers are not managed well in terms of nutrition, estrus detection, etc. Pregnancy rates with low doses of sexed sperm usually are 70-80% of controls with normal numbers of sperm when breeding well-managed heifers.

It is likely that fertility of sexed, frozen sperm at around 2,000,000 sperm/insemination dose would be nearly normal for well managed heifers if the most fertile 30% of bulls were used. We have

not yet done experiments with such selected bulls; our work with lactating dairy cows so far (with bulls unselected for fertility) has involved few animals and has produced mixed results. We have, however, obtained 50% pregnancy (and calving) rates using sexed, frozen sperm to inseminate nursing beef cows (Doyle et al., 2000).

Normality of Calves

Over 2,000 calves have now been born from sperm sorted by sex, and many more are gestating. No gross abnormalities have been observed. To confirm that health and survival rates of calves from sexed sperm are normal, controlled studies involving hundreds of sexed and control calves in similar environments are needed. We have examined pregnancy losses between 1 and 2 months of gestation in some studies; losses were 23 of 261 (8.8%) for sexed pregnancies and 9 of 145 (6.2%) for controls. This small difference is not statistically significant. The actual sexes of calves or fetuses at 2-3 months of gestation, averaged over all studies has been 86% of the desired sex. In our most recent studies, accuracy exceeds 90%. Accuracy is similar for males or females.

While routine freezing of semen kills nearly half the sperm, the process of sexing sperm only damages them slightly. An important fringe benefit of sexing sperm is that the approximately 10% of dead sperm in a typical raw ejaculate (before freezing) are discarded in the sorting process; also, some sperm with abnormal chromosome content are automatically discarded during sexing.

Cost of Sexed Sperm

XY, Inc. intends to license bull studs and other genetics companies to use the technology developed. The sorters themselves currently cost nearly US$300,000 each, although this cost should decline greatly when these machines are simplified and produced in quantity. Since freshly collected semen is required for the sexing process to work well, sorters likely will be placed at bull studs for actual production and distribution of sexed sperm. Only a small portion of each ejaculate is needed to keep equipment for sexing sperm functioning for many hours, so the remainder of the ejaculate can be frozen for routine artificial insemination.

Costs for sexing a low dose of sexed sperm in the United States probably will be in the range of UD$30-50 initially, when thousands of doses are sexed per year. Probably less than UD$20 will be charged when the process is streamlined to produce millions of doses each year. This charge would be in addition to the normal cost of the semen itself.

When Can Sexed Sperm be Purchased?

Large field trials to determine how well sexed sperm works under farm conditions are being done. As in the early days of embryo transfer, fertility of low dose sexed sperm varies considerably, depending on the exact conditions of use. As happened with embryo transfer, procedures are improving rapidly, and success rates likely will stabilize as experience accumulates. Initially, sexed sperm likely will be recommended for use only in heifers; more research is needed for optimal application in lactating dairy cows and nursing beef cows.

Sexed sperm from a few very fertile, genetically elite bulls already are available for purchase in the United Kingdom, and likely will be available soon in several other countries, probably in North America in 2002. The process of negotiating license fees, purchasing and installing complex equipment,

training personnel, and producing and marketing a new product line requires considerable investment in time and money. Cattlemen have looked forward to having this technology for many years, and it will be interesting to see it applied.

Other Methods of Sexing Sperm

Sexing sperm one at a time with a flow cytometer probably will always result in fewer sexed sperm than ideal. Several other methods are being studied, including immunological and electrophoretic approaches. To date, these methods have not resulted in producing offspring of the desired sex. If such procedures would work, many sperm could be sexed simultaneously, thus speeding up the process.

Sexed Sperm in Other Species

Sperm from 6 species of mammals have been sexed by flow cytometry, resulting in offspring: humans, cattle, horses, pigs, sheep, and rabbits. It appears that the process will be successful for nearly all mammals. A major constraint with horses and pigs is that many more sperm are required to obtain reasonable pregnancy rates than with cattle, and since sperm currently are sorted one at a time, procedures using AI of sexed sperm are not as practical as for cattle. We have, however, produced more than 40 pregnancies in horses with sexed sperm to date, and undoubtedly sexing will be commercialized in this species.

There of course is great interest in applying this technology in humans. Fugger (1999) and colleagues have produced hundreds of babies so far with sexed sperm via artificial insemination and in vitro fertilization. An important application is to produce baby girls to avoid X-chromosome-linked genetic diseases.

Implications

Sexed, frozen sperm from a few bulls are likely to become available for purchase within the next year or two. Fertility will be slightly lower than normal with well-managed heifers. Fertility likely will be very variable with poor to average management of heifers, and with well-managed cows. Even with these imperfections and a premium of US$ 30-50 per dose, there will be opportunities for producers to profit from this technology. As with any new technology, improvements will continue to be made, and costs will decline with time.

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Embryonic Development and Factors Affecting Embryo Survival in the Cow

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Key Points

- Pregnancy loss in cattle is concentrated mainly in the first forty days after breeding.
- Endogenous events such as chromosomal abnormalities and hormonal imbalances, as well as environmental factors such as heat stress and nutrition have been implicated as causes of embryonic mortality.
- Hormonal treatments to increase progesterone concentrations, or supplementing the bTP-1 signal may improve pregnancy rates in certain circumstances.

Introduction

The establishment of pregnancy results from the interaction between the embryo and its mother. However, this interaction is the culmination of a series of events initiated with the development of the follicle and the gametes. The endocrine environment and numerous other factors determine whether or not pregnancy will be successfully established and maintained. Embryonic/fetal loss occurs throughout pregnancy in cattle; however, it is concentrated mainly in the first forty days after breeding, in the "embryonic" phase of pregnancy, before the embryo becomes a fetus. Approximately 30% and 40% of the embryonic losses occur by day 7 and by days 8-17 of pregnancy respectively (Thatcher et al., 1994). Pregnancy wastage decreases in severity as pregnancy advances, and fetal loss (after day 42) is generally less than 10% (Roche et al., 1981; Vasconcelos et al., 1997). This article will review some aspects of embryonic development and maternal recognition of pregnancy as well as some of the factors that affect embryonic survival. Embryonic mortality due to infectious agents will not be discussed here.

Early Embryonic Development

In cattle, ovulation (day 0) occurs approximately 30 hours after the onset of standing estrus and fertilization occurs shortly thereafter. The first cleavage of the zygote occurs at about 30 hours post-insemination and the second cleavage follows 10-12 hours later (Hamilton and Laing, 1946). At the 16-cell stage (approximately 100 hours post-insemination), the blastomeres start showing polarity and at the 32-cell stage, compaction is evident. Compaction is very important to the process of formation of the trophectoderm, the inner cell mass and the blastocoel cavity. The inner cell mass is the group of cells that will give rise to the embryo/fetus proper. The trophectoderm is a layer of cells in the periphery of the blastocyst that will give rise to part of the extra-embryonic membranes and plays a role in the formation

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of the placenta. The blastocyst enters the uterus by the 7th day. During the second week, the conceptus elongates and signals its presence to its mother. Attachment of the embryo to the uterine wall is initiated around day 30 in the cow. At day 42, the embryonic period ends and the conceptus is now called a fetus. The major tissues, systems and organs are already formed by that time.

Maternal Recognition of Pregnancy

Maternal recognition of pregnancy refers to the process by which the conceptus signals its presence to its mother. This results in the prevention of the regression of the corpus luteum and maintenance of progesterone secretion which is essential for pregnancy. In the absence of conceptus, in the cyclic cow, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is secreted by the endometrium around day 16. $PGF_{2\alpha}$ causes the secretion of oxytocin from the corpus luteum which in turns causes further increases in $PGF_{2\alpha}$ secretion. $PGF_{2\alpha}$ is responsible for luteolysis in the non-pregnant cow.

In the presence of a conceptus, there is no $PGF_{2\alpha}$ secretion. On days 15-23, the conceptus produces bovine trophoblastic protein-1 (bTP-1) also named interferon-τ (IFN-τ) which reduces the transcription of the gene for receptors to oxytocin and also induces the expression of inhibitors of $PGF_{2\alpha}$ synthesis. It is possible that lower production of bTP-1 by the embryo or failure of the uterus to recognize or respond to that signal is a cause of embryonic mortality. Synthesis of bTP-1 begins around day 15 but only in embryos that are at least 15 mm long (Thatcher et al., 1989). Therefore, embryos that are developmentally delayed may not be able to block regression of the corpus luteum in time.

Other molecules are involved in maternal recognition of pregnancy such as PGE_1, PGE_2, PGI_2 and platelet activating factor. It should be remembered that the conceptus is a foreign body inside the uterus since half of its genome originates from the bull. Therefore, the immune system is involved in maternal recognition of pregnancy by creating conditions that allow the conceptus not to be rejected. Many deleterious cytokines (immune growth factors) such as tumour necrosis factor-α, interferon-γ, interleukin-2 (IL-2) and beneficial cytokines such as transforming growth factor-β, leukemia inhibiting factor, colony-stimulating factor-1, granulocyte macrophage CSF, IL-1, IL-3, IL-4, IL-6, IL-10 appear to be involved in embryo survival in ruminants and other species (reviewed by Martal et al., 1997). It is possible that immunological rejection of the conceptus is a cause of embryonic mortality.

Endogenous Causes of Embryo Mortality

Chromosomal Abnormalities

Chromosomal abnormalities constitute a major cause of embryonic and fetal death. In superovulated cattle, it is estimated that 10% of morulae and blastocysts are cytogenetically abnormal (King, 1990). However, only a few individuals with chromosomal abnormalities are born alive in domestic animals and humans, indicating that most of the chromosomal errors lead to embryonic or fetal death. Chromosomal abnormalities are, either present in the genome of the dam or the sire and are transmitted to a proportion of the gametes; or, they can arise as an error in the process of gamete formation (non-disjunction at meiosis) or at fertilization or in early development.

The best characterized chromosomal abnormality in cattle is the 1/29 Robertsonian translocation which involves a fusion between two nonhomologous chromosomes (1 and 29) which results in a single chromosome. Carriers of this translocation can produce six different types of gametes, only two of which would produce viable offsprings while the other four types would produce chromosomally

unbalanced embryos. Death of these embryos would occur within the 1st or 2nd week of development.

Short Duration of the Luteal Phase

Short-lived corpora lutea were observed in the pubertal heifer and in the postpartum ewe and cow returning to ovulatory activity (Lauderdale, 1986). Moreover, smaller corpora lutea were noted in cows bred at first postpartum estrus (Casida et al., 1968). In fact, during the postpartum period, cows undergo a transition from an infertile to a fertile animal; therefore, all the mechanisms necessary for optimal fertility may not have fully recovered yet. Casida et al. (1968) observed that conception rates in beef cows averaged 15, 55, 64 and 86% for cows inseminated at <31, 31-50, 51-90 and >90 days postpartum, respectively; therefore, the 1st ovulation postpartum may not be fertile in all the cows. It was shown that when the first postpartum corpus luteum began to secrete progesterone, secretion of uterine $PGF_{2\alpha}$ increased earlier than in cyclic cows leading to early luteolysis (Peter et al., 1989; Cooper et al., 1991). Further studies showed that $PGF_{2\alpha}$ itself could have a direct embryotoxic effect and that a factor released by the regressing CL may contribute to embryonic death in the postpartum cow with a short luteal phase (Buford et al., 1996).

Follicular and Oocyte Factors

Embryogenesis begins during oogenesis when the oocyte acquires macromolecules necessary to support early development until the embryonic genome is fully activated. Experiments *in vitro* have demonstrated that oocyte quality (as assessed by visual appearance) has a significant effect on developmental competence. More importantly, oocytes with evidence of advanced degeneration could be fertilized but their developmental competence was drastically reduced compared to oocytes with no or early signs of degeneration (Bilodeau-Goeseels and Panich, unpublished data).

In vivo, oocyte quality is related to the follicular and hormonal environment. Cows with larger preovulatory follicles (12.9 ± 0.9 mm) before the LH surge had lower conception rate (36%) and higher preovulatory concentrations of estradiol than those with smaller follicles (7.5 ± 1.8 mm) which averaged 91% conception rate (Breuel et al., 1993). Under conditions of low progesterone during the estrous cycle before breeding (or low dosages of progestogen when attempting to synchronize estrus), there is increased frequency of LH pulses, a dominant follicle can become persistent and secretion of estradiol is increased. When a persistent follicle ovulates, the oocyte which had been exposed to high concentrations of estradiol could possibly be at a later stage of maturation (Revah and Butler, 1996). As is the case with oocytes with signs of degeneration mentioned above, the ovulated oocyte from a persistent follicle is fertilizable; however, the embryo dies before the 16-cell stage (Ahmad et al., 1995). Fortunately, the presence of a persistent follicle does not alter the developmental potential of oocytes from smaller follicles; that is, if the persistent follicle regresses, normal fertility is expected from the next follicle to develop and ovulate (Smith et al., 1996).

It is now confirmed that cows have two or three follicular waves per estrous cycle. A "wave" begins with the growth of a group of follicles > 4 mm in diameter; however, only one of these develops into a large dominant follicle. Thus, in cows with two or three follicular waves, only the dominant follicle of the 2nd or 3rd wave, respectively, ovulates. The length of the estrous cycle is similar in cows with two or three follicular waves; therefore, in cows with two follicular waves, the ovulatory follicle would be older, and larger and would produce more estradiol than the ovulatory follicle of cows with 3 waves. Given the information mentioned above about persistent follicles, the conception rates might be expected to be lower in cows with 2 follicular waves per estrous cycle. In one study with beef cattle,

conception rates were 82% in cows and heifers in which the ovulatory follicle came from a second wave and 100% in cows and heifers in which the ovulatory follicle came from a third wave of follicular development (Inskeep, 2000). Similarly, in lactating dairy cows, conception rate was reduced when the ovulatory follicle originated from the second (58%) rather than the third wave (95%) of follicular development (Inskeep, 2000). The proportion of animals with two or three waves varies between herds but tends to be repeatable for individual animals. There is evidence that it varies with nutrition (Murphy et al., 1991) and body condition (Burke et al., 1995).

Deleterious effects of excessive secretion of estrogen by a large follicle can also be observed during maternal recognition of pregnancy. Concentrations of estradiol in peripheral blood were measured during days 14 to 17 after breeding in over 100 lactating beef cows. Cows were then divided into three groups according to concentrations of estradiol: the lower quarter, middle half and upper quarter. Conception rate to first service by artificial insemination declined as concentration of estradiol increased. It was then proposed that secretion of estrogen from a large follicle during days 14 through 17 after breeding can compromise embryo survival directly or through interference with the mechanisms of maternal recognition of pregnancy/luteal maintenance (Pritchard et al., 1994).

Exogenous Causes of Embryonic Mortality

Heat Stress

Heat stress is a major factor contributing to low fertility in lactating dairy cows; it is discussed here as this environmental factor can influence several of the key events leading to successful pregnancy (reviewed by Wolfenson and Meidan, 2000). Follicular dynamics are altered during heat stress; more specifically, there is reduction in the dominance of the large follicle. This was found to be associated with an earlier emergence of the dominant/preovulatory follicle of the second wave (Wolfenson et al., 1995). When this follicle ovulates, it would then be older (then the ovulated follicle from non heat-stressed cows) and the duration of dominance of the preovulatory follicle was found to be negatively correlated with fertility (Mihm et al., 1994).

The steroidogenic capacity of follicular cells under heat stress is reduced, primarily due to a drastic reduction of production by theca cells of androgens which serve as substrate for estradiol synthesis by granulosa cells (Wolfenson et al., 1997). Plasma progesterone concentrations are also lowered in cows subjected to chronic heat stress (Howell et al., 1994). A low plasma progesterone concentration affects steroidogenesis in the dominant follicle and in the corpus luteum, causes abnormal oocyte maturation, and leads to altered endometrial morphology and embryo death (Ahmad et al., 1995; Shaham-Albalancy et al., 1996a,b). Oocytes are very sensitive to elevated temperatures and fewer oocytes appear normal during hot periods. However, as development proceeds, embryos acquire a better ability to tolerate heat stress (Edwards et al., 2001).

Nutrition

Nutritional status affects reproductive function in cattle. Poor nutrition results in aberrant estrous cycles, lowered conception rates and reduced birth weight (O'Callaghan and Boland, 1999; Robinson et al., 1999); however, the mechanisms involved remain to be characterized. When heifers were offered high levels of energy intake, an increase in the growth rate of the dominant follicle was observed during the 1st and 2nd follicular wave compared to heifers offered a low level of energy intake (Armstrong et al., 2001). In the same study, an increased nutrient supply (high level of energy intake

and high concentration of dietary crude protein) decreased oocyte quality due in part to increased plasma urea concentrations. Excess rumen degradable nitrogen in ewe diets also elevated urea concentration *in utero* and lead to an increased embryo mortality. Nevertheless, embryo metabolism and fetal growth appeared to be enhanced in the survivors (McEvoy et al., 1997). In high-yielding dairy cows, the detrimental effects of high protein may be mediated by a decrease in uterine luminal pH which, in combination with low progesterone concentration due to a negative energy balance creates a uterine environment that is hostile to the embryo (Elrod and Butler, 1993). Dietary imbalances of specific nutrients such as minerals and vitamins can also disrupt embryo or fetal development (Mills and Delgarno, 1972; Olson and Seidel, 2000; Ross et al., 2000).

A variety of poisonous plants have known detrimental effects on reproduction such as impaired gametogenesis, disruption of ovarian function, embryonic/fetal mortality, reduction in placental function, anatomical abnormalities and neonatal offspring losses (see James et al., 1992 for a list of poisonous plants found in the western rangelands of America which affects reproduction). In some instances, the time when the embryo or fetus is vulnerable to a specific toxin is over an extended period; while in others, the critical window can be as brief as 1 to 2 days (McEvoy et al., 2001).

Prevention of Embryonic Losses

Supplementation with Progesterone

One approach to improving embryonic survival in cattle has been the direct supplementation with progesterone. There are several progestagen preparations commercially available such as Repositol, progesterone releasing intravaginal devices (PRID), controlled internal drug release (CIDR) devices and Syncro-Mate B ear implants. Progesterone treatment seems to increase fertility in herds with low fertility (Robinson et al., 1989; Van Cleeff et al., 1991). However, in one study in which a CIDR-containing progesterone was inserted for 6 or 12 days starting on days 5 to 7, a significantly higher pregnancy rate (79%) was achieved than in untreated cattle even though fertility of the untreated cattle was high (66%) (Macmillan et al., 1991). Injection of progesterone on the first four days of pregnancy appeared to advance development of conceptuses and also to alter secretion of proteins from endometrial tissues at day 5 (Garrett et al., 1988).

Treatments to increase endogenous progesterone production

Any treatment which delays regression of the corpus luteum may allow conceptuses additional time to develop and prevent the secretion of $PGF_{2\alpha}$. Therefore, as an alternative to progesterone treatment, either human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH) were used to increase endogenous progesterone production. Treatment with hCG may slighlty improve fertility in some low-fertility herds such as in heat-stressed cows where conceptuses are slightly delayed in their development (Biggers et al., 1987), and also in embryo transfer recipients (Santo-Valadez et al., 1982). GnRH treatment at the time of insemination had a more consistent effect in repeat-breeder than in first-service cattle in increasing pregnancy rates (Stevenson et al., 1990). An alternative strategy to increase endogenous progesterone concentration is to induce an accessory corpus luteum by ovulating the dominant follicle at day 5 of estrous cycle with hCG.

Supplementation with bTP

As mentioned above, it is possible that a portion of early embryonic deaths in cattle is due to

inadequate secretion of bTP-1 by the conceptus which would not prevent regression of the corpus luteum. Intrauterine injection of recombinant ovine IFN-τ (roIFN-τ) or recombinant bovine IFN-τ (rbIFN-τ) twice daily from day 14 to 24 of the estrous cycle extended corpus luteum lifespan and interestrous intervals and attenuated oxytocin-induced secretion of uterine $PGF_{2\alpha}$ (Meyer et al., 1995). Moreover, neither roIFN-τ nor rbIFN-τ caused a hyperthermic response as was previously observed with IFN-α (Plante et al., 1991). A small percentage of cows may not respond to rbIFN-τ treatment, however, IFN-τ shows good potential as a means of improving fertility in cows.

Implications

Embryonic mortality is a limiting factor to the success of reproduction in cattle. Most pregnancy wastage occurs early during the pregnancy and the causes are usually unknown. Chromosomal abnormalities as well as endocrine imbalances can cause embryonic mortality. Moreover, environmental factors such as heat stress and nutrition have also been implicated. Treatments to increase progesterone concentrations may improve pregnancy rates; therefore, treatments for synchronizing estrus must provide high progesterone and reduce luteolytic influences such as excess $PGF_{2\alpha}$ or estradiol from early after mating until the late embryonic period.

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New Opportunities in Beef Heifer Development

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Key Points

- Heifer selection and management affect herd productivity and profitability but are often given inadequate emphasis.
- Fertility in heifers increases up to the third estrus after puberty.
- Strategic bull exposure or feeding lasolacid may improve reproductive performance.
- Principal advantages of artificial insemination (AI) include using proven calving-ease sires and a shortened calving season.
- Synchronization programs that include either melengestrol acetate or an intravaginal progesterone-releasing implant can result in acceptable fertility to timed-AI in both cycling and prepuberal heifers.
- There are at least two heifer development programs in the United States.
- There is considerable potential for creating a Canadian heifer development and certification program.

Introduction

Although selection and management of replacement beef heifers represents a large investment and substantially affects future productivity of the entire herd, it is often given inadequate attention. On most beef farms, the objective is for heifers to have their first calf at 2-years of age. Since fertility in heifers is modest at the first estrus and reaches a plateau at the third estrus (57 versus 78%, respectively; Byerley et al., 1987), for optimal fertility, heifers should reach puberty concurrent with their first birthday. In general, the probability that a heifer has reached puberty increases with body weight; a common recommendation is that heifers should be approximately 65% of their estimated mature weight at the time of breeding. Heifers that calve early during the first calving season have a higher lifetime productivity than those that calve late. Furthermore, since the interval from calving to first estrus is typically longer in heifers than in cows, it is commonly recommended that heifers are bred at least 2 weeks earlier than cows to allow them a longer interval to resume cyclicity. In addition, the interval

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from calving to resumption of cyclicity will be increased in heifers that are in poor body condition at calving or in those that have difficulty at calving (e.g., hard pull or C-section). Heifers should be fed to be 'fit but not fat'; poor body condition at calving will not reduce the incidence of calving difficulties whereas excessive body condition will increase calving difficulties. The most reliable approach to minimize calving difficulties in heifers is to achieve adequate size and body condition and to use proven calving-ease sires. In summary, a replacement heifer that is grown at an acceptable rate, reaches puberty at approximately 1 year of age, and gives birth (with little or no assistance) to a moderate-sized calf at least 2 weeks before the mature cow herd is expected to have optimal performance and productivity.

Methods to Hasten Puberty

Effects of Bull Exposure

The effect of housing mature sterilized bulls with prepuberal heifers prior to breeding (bull exposure) is dependent upon how close the heifers are to reaching puberty spontaneously. In previous studies, bull exposure failed to affect age or body weight of heifers at puberty (Berardinelli et al., 1978; Roberson et al., 1987), reduced age at puberty (Roberson et al., 1991), or advanced calving date (Makarechian et al., 1985). In a recent study (Small et al., 2000), puberty was advanced in winter-born heifers (close to reaching puberty spontaneously), but delayed in spring-born heifers (several months from spontaneous puberty) when bull exposure was started approximately 1 month after weaning (in the fall). Winter-born heifers exposed to bulls and spring-born heifers not exposed to bulls had the greatest proportion of heifers that exhibited estrus between 10 and 12 months of age and the highest pregnancy rates to timed-artificial insemination (AI). Pregnancy establishment by timed-AI at the beginning of the breeding season resulted in earlier calving and heavier calves at weaning.

Ionophores

The efficacy of lasalocid for heifer development may be dependent upon the proximity of puberty, the quality of the forage, and the duration of the feeding period. The inclusion of ionophores (e.g. lasalocid and monensin) in postweaning diets has reduced age at puberty in heifers (Moseley et al., 1977; McCartor et al., 1979; Goehring et al. 1984) and bulls (Rutter et al., 1991). However, in heifers, advanced puberty did not affect pregnancy rate and the effects of feeding ionophore may have been confounded by the use of sterilized bulls to detect estrus (Mosely et al., 1977; McCartor et al., 1979). More recent work (Small et al., 2000) showed lasalocid slightly reduced growth rate of puberal heifers and slightly enhanced growth rate of prepuberal heifers. Although there was no effect on puberty and no significant effect on body weight or body condition at breeding, both the proportion of heifers responding to prostaglandin treatment and conception rates were enhanced.

Estrus Synchronization and AI

Historically, there has been very limited use of AI in the commercial beef cattle industry. Unfortunately, this represents considerable wasted opportunity. The principle advantages of AI in commercial beef heifers include the ability to use semen from proven calving-ease sires and a shortened calving season. A secondary advantage is that with synchronization programs that include melengestrol acetate (MGA) or an intravaginal implant that contains progesterone (CIDR), heifers that are close to reaching puberty spontaneously will ovulate, with reasonable fertility. In that regard, pregnancy rates to fixed-time AI in a CIDR-based program were 45 and 61% in prepuberal heifers and those that had reached puberty, respectively (Kastelic et al., 2001). However, it must be emphasized that fertility would

likely be low in heifers that were several weeks away from spontaneous puberty (e.g., small, thin heifers). Although it is commonly believed that synchronized breeding will result in all calves being born in a few days, heifers bred on a single day will calve over approximately a 14-day period with a maximum of about 10% of the calves born on any given day.

The economics of natural service versus AI are commonly misunderstood. Using realistic estimates for the costs of purchasing a bull, maintaining him for 3 years, and then sending him to slaughter, we calculated that the annual cost of each bull is approximately $1,000 (Kastelic and Freeze, 2000). Therefore, if each bull is exposed to 25 females, the annual cost per female is $40. Depending on the synchronization program that is used, the cost of AI can actually be less expensive than natural service. The cost of commercial semen varies widely, but in our experience, semen suitable for use in commercial heifers is often in the range of $10 per straw. Furthermore, in one study, we collected semen by electroejaculation, cooled and extended it, and achie· ·d pregnancy rates equivalent to those with frozen-thawed semen (Olson and Kastelic, 2000). It is noteworthy that, based on the number of sperm cells per ejaculate and the number required per insemination dose, a single ejaculate could be used to breed several hundred heifers (perhaps from several herds). Furthermore, instead of purchasing two or three bulls of average genetic quality, producers should consider purchasing a single superior bull, synchronizing females and using fresh-cooled semen for a singled timed-AI, and subsequently using the same bull for natural service to breed those females that were not pregnant to AI. This approach should result in considerable uniformity, as all the calves would have the same sire, with perhaps 60% born a few days apart, and the balance born within the next few weeks.

Although a successful AI program can be conducted without synchronizing estrus, in most cases some means of synchronization is employed to decrease the time and labour needed to detect estrus. When comparing the results of various programs, it is essential to understand the difference between conception rates (percentage of bred cattle that become pregnant) and pregnancy rates (percentage of all cattle eligible for breeding that become pregnant). With timed-AI programs, no estrus detection is done and all heifers are bred at a fixed-time; therefore, the conception rate and the pregnancy rate are the same. In contrast, if heifers are only bred following detection of estrus, then the proportion of heifers detected in estrus limits the pregnancy rate. For example, if 80% of the heifers have reached puberty, 90% of puberal heifers respond to treatment and are in estrus, 80% of those are detected in estrus and the conception rate is 65%, then the pregnancy rate will be only 37%. Although timed-AI programs inevitably use more semen than programs in which heifers are bred following detection of estrus, time and labour inputs are usually lower, and pregnancy rates usually are comparable or higher than programs that rely on estrus detection.

Prostaglandins

Although prostaglandins have been commercially available in Canada for approximately 25 years, in many cases they were used inappropriately with (predictably) low pregnancy rates. Prostaglandins cause luteolysis (regression of the corpus luteum) when given approximately 6 to 17 days after estrus. When treatment is given very soon after estrus, luteolysis is unlikely to occur. If treatment is given late in the cycle (i.e., after about Day 17), the heifer will have an endogenous prostaglandin release that will cause luteolysis. Therefore, prostaglandins are effective only in heifers that are cycling and only during about 60% of the cycle. Furthermore, in the absence of any treatment to synchronize the growth and ovulation of ovarian follicles, the interval from prostaglandin treatment to estrus can range from approximately 2 to 6 days (Kastelic et al., 1990). Therefore, it is not surprising that many

prostaglandin-based programs in heifers resulted in poor pregnancy rates (Day, 1998), particularly if heifers were bred at a fixed-time (without estrus detection).

Several prostaglandin-based programs have been developed for cattle (Day, 1998). For example, heifers can be examined by rectal palpation or ultrasound; those judged to have a responsive corpus luteum are treated immediately and those that are cycling but currently lack a responsive corpus luteum are treated approximately 10 days later. The success of this approach is largely dependent upon the skill of the examiner. Alternatively, two doses of prostaglandin can be given 11 or 12 days apart and heifers either observed for estrus and bred after both treatments (those bred after the first treatment are not retreated) or observed for estrus and bred only after the second treatment. Finally, heifers can be observed for estrus for 5 or 6 days; those detected in estrus are bred and those not detected in estrus are given prostaglandin on Day 5 or 6 and observed for an additional 5 days (and bred after detection of estrus). If the proportion of heifers detected in estrus after the first 5 or 6 days is substantially less than 20 to 25%, then there is either a problem with estrus detection and(or) many of the heifers have not reached puberty; in this case, it is wise to examine (rectal palpation) at least some heifers to determine whether they are cycling. This program works very well if a high percentage of the heifers have reached puberty and there are adequate resources to detect estrus and AI heifers over a period of approximately 11 days.

Melengestrol Acetate (MGA) for Estrus Synchronization

Although MGA is licensed only for suppression of estrus in beef heifers, it has also been used for synchronization of estrus. The advantages of MGA include very low cost and toxicity, and oral administration. It is important to ensure uniform intake on a daily basis. Therefore, thorough mixing and uniform intake of the feed containing MGA are essential. Although MGA is used most commonly in heifers under feedlot conditions, it could be used in pastured heifers if they were accustomed to being supplemented.

In historical studies (Patterson, 2000), MGA was fed for prolonged periods (e.g., 17 to 20 days). Although estrus was detected in a majority of the cattle within a few days after the last feeding, fertility was poor (conception rates of approximately 30%). It was subsequently determined that, in the absence of a functional corpus luteum, large, persistent follicles developed in MGA-treated cattle, and, following withdrawal of MGA, ovulated an 'aged' oocyte that had poor fertility (Patterson, 2000). It is noteworthy that fertility was poor only for the estrus period following MGA withdrawal; fertility at the next estrus period was normal. Therefore, in an alternative regimen, MGA was fed for 14 days and prostaglandin given 17 days after the last feeding. This resulted in reasonable synchrony with acceptable fertility (conception rates of approximately 50 to 60%) if cattle were bred following detection of estrus (Patterson, 2000). It was subsequently shown that giving prostaglandin 19 days after the last feeding of MGA further improved estrus synchrony (Patterson, 2000). To overcome the long interval from the start of the treatment to breeding, another approach was to feed MGA for short intervals (7 to 9 days), with prostaglandin given at the time that MGA was discontinued. In these studies, conception rates were approximately 40 to 50%; it was subsequently shown that cattle that were not cycling or late in the estrus cycle at the time that MGA was fed developed persistent follicles and had poor fertility. Therefore, this regimen was modified by giving either gonadotropin releasing hormone (GnRH) or estradiol and progesterone at the start of the treatment to cause regression of large follicles and synchronize the emergence of a new follicular wave (Thundathil et al., 1999). This resulted in acceptable synchrony and fertility (conception rates of 55 to 60%). A further modification is to give either GnRH or estradiol to

synchronize ovulation, with acceptable pregnancy rates (approximately 55 to 60%) to timed AI (Martinez et al., 2001a).

Progesterone for Estrus Synchronization

A Controlled Internal Drug Release (CIDR) device containing progesterone that is placed within the vagina has been successfully used in New Zealand for many years. Following several years of testing in North America, this device was very recently approved for use in beef cattle in Canada. The adoption rate of this device has been extremely rapid, with a substantial number of the devices sold for synchronizing estrus in beef cattle (both cows and heifers). The devices are placed in the vagina for 7 or 8 days, with treatments (usually either GnRH or estradiol) given both at the time of device insertion (to synchronize wave emergence) and after device removal (to synchronize ovulation). Pregnancy rates to timed-AI in heifers synchronized with CIDRs are typically 55 to 65% (Martinez et al., 2000; Martinez et al., 2001b).

Heifer Development Programs in the USA

Although numerous technologies and management strategies have been developed for raising replacement heifers, few farms have comprehensive programs. The authors are aware of two heifer programs currently underway in the United States.

Missouri has a state-sanctioned heifer certification program (Missouri Show-Me-Select Program; http://agebb.missouri.edu/select/index.htm) for both open and bred heifers. There are several criteria that must be met for heifers to be included. Open heifers must be home-raised or purchased prior to 270 days of age, whereas bred heifers must be owned at least 60 days prior to breeding. Purchased heifers must have documentation of their previous owner and approximate date of birth. There are specific health requirements, including the diseases that must be vaccinated against and the timing of vaccine administration, as well as treatment for internal and external parasites. Furthermore, heifers must meet minimum requirements for frame score, weight, body condition, pelvic area, and disposition. Bred heifers must have been serviced by bulls of known identification and breed, with complete expected progeny differences (EPD) information, with specific requirements for calving ease. Heifers that meet all the requirements are given a special program ear tag and an official health certificate and are typically sold at specific auction sales featuring only these heifers.

A similar heifer development program has been developed in Minnesota (SW Minnesota Beef Heifer Development Program). This program has been conducted on a small scale at one custom feedlot that accepts heifers soon after weaning and retains them until after they are bred. Heifers must meet minimum requirements for vaccination and health status, size, weight, pelvic area, and disposition. Heifers that fail to meet minimum requirements can either be returned to their owner or fed for slaughter. A recent addition to the health status is that all heifers must have a negative blood test for the presence of BVD virus (to ensure that no persistently affected heifers enter the feedlot). Since the prevalence of infected cattle is generally very low, in some herds, blood samples from several heifers are pooled prior to analysis; in the case of a positive test, then the test is repeated individually on all heifers in that pool. In this program, heifers are synchronized and bred exclusively by AI. Owners are charged set fees for feed and yardage, synchronization, breeding, etc. After breeding heifers are returned to their owners and either retained as replacement heifers or offered for sale.

Canadian Heifer Development and Certification Programs

The authors are not aware of any formal heifer development programs in Canada at the present time. However, in a few feedlots in southern Alberta, there appears to be an emerging niche industry of purchasing weaned heifers, backgrounding them through the fall and winter, breeding them (usually AI) to proven calving-ease sires in the spring, sending them to summer pasture, and then marketing them in the late fall or early winter.

The authors propose that there would be substantial merit in developing a heifer development and certification program in Canada, based on the programs in Missouri and Minnesota. With input from the cattle industry and persons with expertise in management, nutrition, reproduction, and infectious disease, specific criteria relevant for our conditions could be determined. The overall objectives of this program would be to serve as a repository of information related to heifer management as well as a program that would certify heifers for sale. Therefore, the information could be used both by producers raising their own replacement heifers on a retained-ownership basis as well as those raising replacement heifers for sale. Defining relevant criteria, providing accurate information and options regarding management, and developing a trustworthy certification system will undoubtedly improve the quality of replacement heifers that are produced and be of considerable benefit to the cattle industry (and to allied industries).

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